EVALUATION OF SOME INTRODUCED FRESH MARKET TOMATO (*Solanum lycopersicum* L) FOR GENETIC VARIABILITY AND ADAPTABILITY IN GHANA USING MORPHOLOGICAL AND MOLECULAR MARKERS.

BY

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JUNE, 2014

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SCHOOL OF GRADUATE STUDIES

DEPARTMENT OF CROP AND SOIL SCIENCES

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THESIS SUBMITTED TO THE DEPARTMENT OF CROP AND SOIL SCIENCES, FACULTY OF AGRICULTURE KNUST, KUMASI

IN PARTIAL FULFILMENT OF THE REQUIRMENT FOR THE AWARD OF

M.Sc. (PLANT BREEDING)

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JUNE, 2014

DECLARATION

I hereby declare that this work is a direct result of my field and laboratory research undertakings and are supported by cited references in relation to other previous and similar work performed, and therefore this thesis has not been presented anywhere for a degree.

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ABSTRACT

The tomato sector in Ghana has failed to realize its full potential, in terms of attaining yields comparable to other countries, sustaining its processing plants and improving livelihoods of those households involved in tomato production. The need therefore, to evaluate some introduced tomato varieties in order to identify varieties with good attributes and make recommendation to tomato farmers cannot be overemphasized. This study thus looked at some introduced tomato varieties from the USA for adaptability to environmental conditions in Ghana with focus in the Ashanti region. Field experiments were conducted at the Department of Horticulture, KNUST and Horticulture Division of CSIR – Crops Research Institute (CRI), Kwadaso. Four tomato varieties from the USA and a local check from CSIR-CRI, Kumasi, Ghana were planted to plots of 264 m^2 (12 m x 22 m). Data were taken as soon as plants were established on the fields. These included number of days to flowering (1^{st}, 50%, 100%), plant height, stem length, stem diameter, number of leaves under 1^{st} inflorescence, number of flowers per inflorescence, growth habit, number of plants harvested, number of marketable and non- marketable fruits, average weight of marketable fruits, total marketable fruit weight and yield (tonnes per hectare), fruit shape, disease and insect pest incidence, post-harvest characters such as brix and shelf life. A sensory evaluation was carried out at CRI, Kwadaso. A total of twenty volunteers participated in a sensory test. Selected tomato fruits from each variety were used in preparing soup, stew and salad. Accessions were labeled as 100, 200, 300, 400, and 500 without displaying the actual variety names to the participants. They were scored for taste, flavour, sweetness, appearance, colour and mouth-feel. Additionally, PCR analysis was done using fifteen (15) SSR primer pairs obtained from Metabion International Laboratory, Germany to determine the diversity existing among the tomato varieties. Agronomic characters of the tomato varieties showed significant differences in almost all the genotypes across locations. The highest average plant

height at both locations was recorded on Shasta (KNUST-98.2 cm and Kwadaso-84.1 cm). Likewise varied differences were also found on the number of inflorescence per plant and flowers per inflorescence. The tomato varieties showed one to two growth patterns; determinate or semi determinate. Low disease infections and pest pressure were observed during the growing season and this may be due to good agronomic practices carried out on the field. On the sensory evaluation test, significant differences were only found in the soup and stew preparations on their appearance and flavour respectively. This was evident on OP-B149, Shasta for the soup appearance and Heinz, OP-B155 for stew flavour. The 15 SSR markers used for genetic characterization revealed substantial genetic variation among the tomato varieties studied. Based on yield (t/ha), number of plants per plot (establishment), marketable fruits, average fruit weight, plant height and shelf life, Shasta and CRI-POO were rated high in terms of adaptability.

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LIST OF ABBREVIATIONS

A	Adenine
AFLP	Amplified Fragment Length Polymorphism
AGE	Agarose Gel Electrophoresis
AVRDC	Asian Vegetable Research and Development Center
bp	Base pair
BER	Blossom End Rot
BW	Bacterial Wilt
C	Cytosine
CRI	Crops Research Institute
CSIR	Council for Scientific and Industrial Research
CTAB	Cetyl Trimethyl Ammonium, Bromide
cm	Centimeter
COS	Conserved Orthologue Set
DNA	Deoxyribonucleic acid
DNTP'S	Dinucleotide Triphosphates
DArT	Diversity Array Technology
EDTA	Ethylene Diamine Tetracetic Acid
EB	Early Blight
EST	Expressed Sequence Taq Polymorphism
FAO	Food and Agriculture Organization
FW	Fusarium Wilt
IBPGR	International Board Plant for Genetic Resources
IPCC	Intergovernmental Panel on Climate Change
Kb	Kilobases (lkb=103 base pair)

L	Liter
LB	Late Blight
MOFA	Ministry of Food and Agriculture
MABC	Marker-Assisted Backcrossing
MAS	Marker-Assisted Selection
$MgCl_2$	Magnesium Chloride
PCR	Polymerase Chain Reaction
Pj	Rate of Polymorphism
q	Allele Frequency
QTL	Quantitative Trait Loci
RAPD	Random Amplified Polymorphic DNA
RFLP	Restriction Fragment Length Polymorphism
RCBD	Randomized Complete Block Design
SAHN	Sequential, Agglomerative, Hierarchical and Nested
SNP	Single Nucleotide Polymorphism
SSR	Simple Sequence Repeats
TGRCC	Tomato Genetic Resources Center in Califorina
TYLCV	Tomato Yellow Leaf Curl Virus
TSWV	Tomato Spotted Wilt Virus
ToMV	Tomato Mosaic Virus
Taq	Thermus Aquaticus
TAE	Tris-Acetate-EDTA buffer

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the study

Tomato (*Solanum lycopersicum L*) is an edible fruiting plant often classified as a vegetable. It is known to have originated from Latin America (Yamaguchi *et al.*, 1983; Harlan, 1992). In Ghana, it is an essential ingredient in the diet of many people. It is used in preparing a variety of foods such as soups, sauces, stews, salads and other dishes. In Ghana, tomato is normally used in large quantities as compared to other vegetables (Ellis *et al.*, 1998). The fruit is fairly nutritious and contains high amounts of vitamins A and C (AVRDC, 2004). Tomatoes can be produced across a wide range of soils as long as drainage and the physical soil structure is good. However, it does very well in well-drained sandy loams to loamy soils with optimum pH range of 6.0 - 6.5. In Ghana, highest quality fruits and greatest yields are obtained in the dry season with supplementary water (Osei *et al*., 2010). Tomato is the most important vegetable in the tropics (FAO, 1990; 2003). Universally, it is the second most consumed vegetable after potato (FAOSTAT 2005, Osei *et al.*, 2010). The current world production of tomato is about 100 million tons of fresh fruit produced on 3.7 million hectares (Osei *et al*, 2010). In Ghana, the average yield on a farm is low between 7.5-10 mt/ha (Osei *et al.*, 2010). Presently, more money is used on tomato than on any other vegetable (Osei *et al.*, 2010). According to Osei (2010), usually vegetables account for 9.6% of the entire food expenditure and 4.9% of the whole expenditure of Ghana. Tomato makes up to 38% of the vegetable outflow.

In spite of its importance in Ghana, local production of tomato is not able to meet the domestic demand. Some farm yields are as low as 7.5-10 t/ha (Osei *et al*., 2010). All available evidence suggests that seed quality can influence yields. Some introduced varieties in Ghana are not up to the satisfaction of growers. Additionally, majority of these varieties are introduced with no formal testing. This sometimes results in disease outbreaks, unavailability of seeds, poor adaptability, among other things. The need, therefore, to evaluate introduced tomato varieties to identify those with good attributes and make recommendations to tomato farmers cannot be overemphasized. This study therefore looked at some introduced tomato varieties from the USA for adaptability to environmental conditions in Ghana with focus in the Ashanti region. Further, improvement programmes of evaluation are very important, to understand the genetic background and breeding value of existing tomato plants. Numerous research findings stress on the agronomic, morphological and biochemical parameters that have been widely used in the assessment of various crops (Osei *et al.*, 2014). Morphological traits are important diagnostic features for distinguishing genotypes. These distinct morphological traits of genotypes facilitate the selection process in crop improvement by serving as genetic markers. The normal approach to characterization and evaluation of populations involves cultivation of sub-samples by assessing their morphological and agronomic description (Perez de la Vega M. 1993; Osei *et al*, 2014). Manipulation of such traits increases understanding of the genetic variability available which facilitates breeding for wider geographic adaptability, with respect to biotic and abiotic stresses. Also, gene diversity needs to be shown and measured if it is to be successfully integrated into breeding strategies and management of plant genetic resources. The identification of variability among accessions is vital to the maintenance, utilization and acquisition of germplasm resources (Mwirigi *et al.* 2009). The International Plant Genetic Resources Research Institute (IPGRRI) has developed descriptors for

quantitative as well as qualitative characters to ensure precise, accurate and uniform identification of genotypes (Chavez 1990).

The main objective of the work was:

- To provide farmers with better and superior varieties of tomatoes.

The specific objectives were to:

- To identify high yielding tomato varieties that are adaptable to the local conditions.
- To identify tomato variety that is resistant to virus related disease.
- To select tomato variety that has good postharvest and sensory characteristics.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Origin and domestication of Tomato

Tomato *(Solanum lycopersicum)* originated from the Andean region which now includes parts of Chile, Boliva, Ecuador, Colombia and Peru. The original site of domestication and the early events of domestication remain unclear (Peralta and Spooner, 2007). Two hypotheses have been advanced for the original place of tomato domestication; one hypothesis proposes Peru while the other proposes Mexico (Peralta and Spooner, 2007). Though definite proof for the time and place of original domestication is lacking, Mexico is widely assumed to be the most probable region of domestication, with Peru as the center of diversity for wild relatives (Larry and Joanne, 2007). *Solanum lycopersicum cerasiforme* is thought to be the ancestor of cultivated tomato, based on its wide presence in Central America and the presence of a shorten style length in the flower (Cox, 2000). However, recent genetic investigations have shown that the plants known as 'cerasiforme' are a mixture of wild and cultivated tomatoes rather than being 'ancestral' to the cultivated tomatoes (Nesbitt and Tanksley, 2002). The genetic difference present in the wild species has been examined for precise traits and is being exploited in tomato breeding (Walter, 1967; Rick and Chetelat, 1995; Larry and Joanne, 2007). Associated with the rich pool in wild species, the cultivated tomato is genetically poor. It is estimated that the genomes of tomato cultivars contain less than 5 % of the genetic variation of their wild relatives (Miller and Tanksley, 1990). The lack of diversity in the cultivated tomato can be visualized using DNA technologies. Few polymorphisms within the cultivated tomato gene pool have been recognized, even using complex molecular markers (Van der Beek *et al.*, 1992; Villand *et al.*, 1998; Park *et al.*, 2004; Garcia-Martinez *et al.*, 2005; Tam *et al.*, 2005). In addition to the

genetic resources preserved in the gene banks, wild tomato relatives still grow in the center of origin of tomato from the northern part of Chile to Colombia. In 2005, two new species of wild tomatoes (*S. cheesmaniae* and *S. pimpinellifolium)* were identified from Peru (Peralta *et al.*, 2005). Tomato had reached an advanced stage of domestication before being taken to Europe in the 15th century and further domestication on a much stronger level arose throughout Europe in the 18th and 19th centuries (Sims, 1980). It was introduced in the West African sub-region by the Portuguese between the sixteenth and seventeenth Century (Norman, 1992), and has since become the most popular vegetable crop (Norman, 1992; Nkansah *et al.*, 2003; Osei *et al*., 2010). In Ghana it is now the number one vegetable consumed (Schippers, 2000; Osei *et al.*; 2010). Its cultivation provides a major source of employment to many in the country, especially in the Ofinso North, Bolga and Ada districts (Norman, 1992).

2.2 Classification by fruit type

The commonly grown types of tomato are the cherry, plum (Roma), and the shared table varieties (Jansen and Shock 2009). Relf *et al.*, (2009), on the other hand, categorize tomatoes based on their fruit characteristics such as the cherry tomatoes, beefsteak type tomatoes, paste tomatoes, winter storage tomatoes and tomatoes categorized by the colour of their fruit. In Ghana, there are many varieties of tomato grown in different regions and under various conditions. According to MOFA (2008), commonly grown varieties of tomato in Ghana are the Roma VF, Pectomech VF, Tropimech, Rio Grande, Cac J, Wosowoso, and Laurano 70. Robinson and Kolavalli (2010) describe the Pectomech variety as being appropriate for processing, being favored by consumers and realizing the best price over other varieties. Adubofour *et al.* (2010) also quoted two varieties of tomato grown in Ghana as the Bolga and Ashanti. Robinson and Kolavalli (2010) however, pronounced Power Rano and "No name", often grown in rain-fed environments in Brong Ahafo and under irrigated condition in the

Upper East Region as other varieties. Lastly, Robinson and Kolavalli (2010) identified main local open pollinated varieties such as Rasta, Power, Power Rano, and Wosowoso, with Power Rano often being chosen due to its high tolerance and/or resistance to diseases. Ellis *et al*., (1998) describe the `Power' variety as the predominant variety for cultivation in Ghana.

2.2.1 Economic Importance of tomato.

The cultivated tomato, *(Solanum lycopersicum)* is the third most variable of all crop species and the most appreciated in terms of vegetable crops (van der Hoeven *et al.*, (2002). It is usually grown around the biosphere and establishes a major agricultural industry. Universally, it is the second most paid vegetable after potato (FAOSTAT 2005) and conclusively the most general garden crop. In the U.S., it is the third most economically important vegetable (with a total farm value of $2.062 billion) following potato ($2.564 billion) and lettuce ($2.064 billion) (USDA 2005). In addition to its consumption as a raw vegetable or being added to other food items, tomato may be processed into a variety of foods including paste, whole peeled tomatoes, diced products, and numerous forms of juice, sauces, and soups. In 2013, the total harvested area in the U.S. was estimated to be 430,000 ha (130,000 ha fresh market and 300,000 ha processing tomatoes) with a total farm value of about $3.00 billion ($1.6 billion fresh market and $1.4 billion processing) (USDA 2013). Pennsylvania is by far the leading producers of processing and fresh market tomatoes, respectively (USDA 2013). Universally, tomatoes are a significant part of a diverse and balanced diet (Willcox, *et al.,* 2003). In Ghana, tomato forms a very essential part of human food (Beecher, 1998; Osei *et al*., 2008) and is consumed in diverse ways. It is consumed as raw food and as an essential ingredient in many dishes, sauces, and drinks (Alam *et al*., 2007, Tambo and Gbemu, 2010, Yeboah, 2011). Therefore, Lycopene is the pigment principally responsible for the characteristic deep-red color of ripe tomato fruits and tomato products (Shi *et al.* 2000). It has attracted attention due to its biological and physicochemical

properties, especially related to its effects as a natural antioxidant (Shi *et al.* 2004). Tomatoes and related tomato products are the major source of lycopene compounds, and are therefore considered as an important source of carotenoids in the human diet (Willcox, *et al.,* 2003). The tomato products are a superior source of alpha-tocopherol and vitamin C (USDA 2004). In addition to their micronutrient benefits, tomatoes also contain valuable phytochemicals, including carotenoids and polyphenols (USDA 2004).

2.2.2 Adaptability of tomato

Tomato (*Solanum lycopersicum* L.) production is higher than other vegetable crops in Ghana, with a total annual production of 350,000 tonnes, representing 38% of the total vegetable production (Osei *et al.,* 2010). Tomato production in Ghana covers mainly the Upper East, Eastern, Ashanti, Brong Ahafo and Greater Accra regions (Yeboah, 2011). Low production is caused by a mixture of factors including abiotic stress such as salinity, drought, excessive heat, declining soil fertility and biotic stress such as pests and diseases. Increasing productivity will require development and introduction of new varieties that are adaptable to specific environment. In other countries, some varieties are available with resistance to diseases such as Tomato yellow leaf curl virus (TYLCV), bacterial wilt (BW), fusarium wilt, gray leaf spot, early blight and nematodes (Cornell University, 2006). However, only a few of these introduced varieties are adapted in Ghana (Yeboah, 2011).

2.2.3 Post harvest and Sensory characteristics of tomato.

Consumers have complained about tomato flavour for more than ten years in several countries of Europe (Decoene, 1995; Janse and Schols, 1995) as well as in the USA (Kader *et al.,* 1977) and Australia (Ratanachinakorn *et al.,* 1997). Hobson *et al.,* (1990) characterized the flavour of diverse tomato varieties by sensory prowling and presented the potential of cherry tomato, which has sweeter fruits and higher overall aroma intensity than large-fruited

tomatoes. The flavour of tomato is influenced not only by varietal modifications and the nutrition of the plants (Hobson and Bedford, 1989; Petersen *et al.*, 1998), but also by the stage of ripening when picking fruit (Kader *et al.*, 1977, 1978) and through post-harvest storage conditions (Ratanachinakorn *et al.*, 1997). Sensory analyses showed that sweetness and sourness are the major determinants of tomato flavour preference (Stevens *et al.*, 1977). Moreover, changes in texture (Harker *et al.*, 1997), and increase in the firmness of modern varieties are some consumers' complaints. Numerous relationships were found amongst tomato fruit composition (Davies and Hobson, 1981), physical characteristics, and sensory traits (Baldwin *et al.*, 1998). Moreover, 400 aroma volatiles were recognized in tomato fruit (Petro-Turza, 1987), though, they are not all equally significant to the development of tomato aroma (Baldwin *et al.*, 1998; Krumbein and Auerswald, 2004). Variety (Langlois *et al.*, 1996), ripening stage (Baldwin *et al.*, 1991) and storage conditions (Stern *et al.*, 1994) may influence the content of aroma volatiles, but little is known about their genetic control (Stevens, 1986) and the genes accountable for their variation.

2.2.4 Plant Spacing and Density

The object in a tomato production is to utilize all the growing space to maximize light interception and provide sufficient space between rows to service the plants. However, there is an ideal plant spacing pattern best suited for every growing system. The extent of shading can be varied by altering the plant spacing pattern (http://www.growtomatoes.com/tomato-cultural-practices/). With the physical arrangement of plants being normally in double rows, the space between the rows and the plant spacing within the row can significantly affect light penetration through the canopy (http://www.growtomatoes.com/tomato-cultural-practices/). In a plant spacing of 2.5 plants per square meter in a plant spacing configuration of 45 by 45 cm, about 60-70% of the solar radiation during the 1-hour period of solar noon will reach the

floor. By increasing the plant spacing to 3.5 plants per square meter, 60-70% of the solar flux is intercepted by the plant canopy. Suggested optimum spacing of plants in double rows at 80-cm spacing within the row and 1.2 meters between the double rows {http://www. growtomatoes.com/tomato-cultural-practices}

2.2.4.1 Planting Schedules

A two-crop season is the common practice in the western latitudes in order to avoid the high light intensity months (January - March). Plants are set in late May and harvesting of fruit continues through August, and then plants are set in September and harvesting of fruit continues into December or January. In the lower latitudes where the period of cold and low light intensity is less, a single crop is commonly used with plants set in May and the crop is carried through until July or August. In Colorado greenhouses, a staggered schedule of transplanting and replacing plants every 8 months in a two-row planting system ensures continuous production of fruit year-round. Variations of these various planting schedules are practiced based on consumer demand and price obtained for fruit http://www.growtomatoes .com/tomato-cultural-practices.

2.2.4.2 Water Management

Tomatoes require a constant supply of moisture throughout the growing season. In field situations, tomatoes require 214-706 thousand gallons/ac of water per season to produce a high yielding crop {http://www.yara.us/agriculture/crops/tomato/key-facts/agronomic-principles/default.aspx}. Excess water, however, leads to root death in anaerobic soil conditions, as well as delay, less prolific flowering and fruit set. Too much water after fruit set induces several fruit disorders, most notably cracking. Flowering is also adversely affected under conditions of moisture stress. Blossom end rot (BER) can also result in the

absence of water coupled with calcium deficiency {http://www.yara.us/agriculture/crops/tomato/key-facts/agronomic-principles/default.aspx}.

2.2.4.3 Crop Protection

Plant breeders are increasingly introducing varieties that are resistant to or offer partial resistance to a range of diseases such as Verticillium wilt, Septoria, Fusarium, Alternaria, Stemphylium and tobacco mosaic virus, as well as nematodes. Growers can minimize the impact of diseases such as blight by using appropriate fungicide programmes. Integrated Crop Management techniques that keep the foliage dry, and dew free, will help minimize outbreaks of diseases such as Blight. Sterilization of soils, hot water treatment of seeds and appropriate use of bactericides will minimize bacterial canker and bacterial spot. Insect pests such as whitefly, thrips and red spider mite are more difficult to control as a result of increasingly widespread resistance to pesticides. In soil grown crops, weed control is essential to reduce competition for moisture and nutrients {http://www.yara.us/agriculture/crops/tomato/key-facts/agronomic-principles/default.aspx}.

2.2.5 Biotic and abiotic stresses of tomato

Most plants grow in environments that are suboptimal, which prevents the plants from achieving their full genetic potential for development and reproduction (Bray *et al.*, 2000; Rockstrom and Falkenmark, 2000). The harvest differences can mostly be explained by unfavourable ecological conditions, which, after causing potentially damaging physiological changes within plants, are recognized as stresses (Shao *et al.*, 2008). Additionally, plants must protect themselves from attack by a great variety of pests and pathogens, including fungi, bacteria, viruses, nematodes, and herbivorous insects (Hammond-Kosack and Jones, 2000). Each stress causes a compound cellular and molecular response system applied by the plant in order to avert damage and sustain existence, but frequently at the expense of growth

and yield (Herms and Mattson, 1992). Moreover, plant responses to necrotrophic fungi are complex, involving various genetic and molecular mechanisms, and depending on the main mechanism of the pathogen virulence (Wolpert *et al.*, 2002; Glazebrook, 2005). Wide host necrotrophic fungi produce toxins, cell-wall degrading enzymes and reactive oxygen intermediates that determine the severity of disease (Edlich *et al.*, 1989; Tiedemann, 1997; Muckenschnabel *et al.*, 2002). These disease factors cause electrolyte leakage, changes in ion fluxes, cell death and other stress responses, relating to the plant's responses to microbial necrotrophy and abiotic stresses. According to Zhang *et al.*, (2003), Early Blight (EB) is one of the most shared and negative pathogen of tomato, causing symptoms throughout the plant and in all phases of development. The late blight on tomato caused by Phytophthora infestans (Mont.) deBary is a common disease which occurs in cool and moist areas, producing large damages in field and in green-houses (Moreau *et al.*, 1998). Tomato spotted wilt virus (TSWV) contributes to a significant yield and fruit quality decline due to the presence of necrotic or chlorotic spots on fruits (Paterson *et al*. 1989). Mosaic of tomato caused by tomato mosaic virus (ToMV) is a major disease and leads to heavy losses in tomato production (Ohmori *et al*., 1996). Tomato yellow leaf curl virus belongs to begomoviruses and is transmitted by whitefly. It manifests itself in chlorosis between the veins of leaves and causes the distortion of young leaves and reduced plant growth (Pico *et al*. 1996). Abiotic stress is caused by heat, cold, drought, salinity and nutrients; these have a great influence on agriculture. They reduce yields by more than 50% (Wang *et al.*, 2003). Present climate forecast models show that average temperatures will rise by 3–5 °C in the succeeding 50–100 years, severely distressing agricultural systems worldwide (IPCC, 2007). This will increase the occurrence of drought, flood, and heat waves (IPCC, 2008; Mittler and Blumwald, 2010). Changes in rainfall and temperature may affect yields as well as the quality of crops (Porter and Semenov, 2005). Temperature changes affect the environment, a variety of pests and

pathogens; an increase in temperature facilitates pathogen spread (Bale *et al.*, 2002; Luck *et al.*, 2011; Madgwick *et al.*, 2011; Nicol *et al.*, 2011). Plants that experience a larger range of environmental stresses usually have severe consequences of reduce yield.

2.3 Measurement of tomato diversity

The breeding system was extensively considered, using the clade as a standard to study its properties on species variation (Bedinger and Chetelat, 2011). Finally, the species of the clade are inter-crossable, but with a changeable success rate (Rick and Fobes, 1977a; Rick and Fobes, 1979). This background has realized the development in public institutes of plant germplasm banks. The original idea for collecting and preserving current genetic diversity was derived from the pioneer work of Nikolai Vavilov (1887-1943) (Kurlovich and Repev, 2000). Later on, he was followed by Charles Rick (1915-2002) who devoted his life to discovering, collecting and characterizing exotic tomato germplasm (Tanksley and Khush 2002). Today, more than 83,000 tomato accessions are deposited in seed banks worldwide. It holds first position in this regard among vegetable species collected (FAO 2010). The main collections in the world are: In USA, the Tomato Genetic Resources Center in California (TGRC), (www.tgrc.ucdavis.edu) and the USDA collection (www.ars.usda .gov), the World Vegetable Center in Taiwan (www.avrdc.org) and several Europeans collections. The establishment of tomato resource collections made major contributions to understand the distribution of its diversity around the world including Ghana.

2.3.1 Morphological characteristics of Tomato

Morphological characters have for a long time remained the means of studying genetic variations in plant species. Morphological data are affected by ecological interactions; thus explanations must be made with suitable replication (Beckmann & Soller (1986). Valid comparisons are only possible for accounts taken at the same location and during the same

season (Smith & Smith, 1988). Beckmann & Soller (1986) stated that related to morphological and biochemical characteristics, the DNA genome provides a meaningful and more influential source of genetic variances. Phillipp *et al.,* (1994) also pointed out that the benefit of DNA fingerprinting over morphological markers is the dominance and the lack of pleiotropic effects. Among these morphological properties, length, width, thickness, mass, volume, projected areas and center of gravity are the most important factors in sizing systems (Mohsenin, 1986). Stroshine and Hamann, (1995) assert that the association between weight and major, minor, and intermediate, diameters is needed. Determining relations between mass and dimensions and projected areas may be beneficial and applicable (Stroshine and Hamann, 1995).

2.3.1.1 Characteristics of molecular markers.

A molecular marker is a DNA sequence that is readily sensed and whose heritage can be easily observed (Weising *et al.,* 1995). The use of molecular markers is based on the naturally occurring DNA polymorphism, which forms the basis for manipulative approaches to explore for practical purposes. According to Weising *et al.,* (1995), an ideal molecular marker must have some desirable properties such as 1) highly polymorphic: It must be measured for genetic diversity studies. 2) Co-dominant inheritance: determination of homozygous and heterozygous states of diploid organisms. 3) Numerous occurrences in genome: A marker should be evenly and regularly circulated throughout the genome. 4) Selective neutral behaviours: The DNA sequences of any organism are neutral to environmental conditions or management practices. 5) Easy access: It should be easy, fast and cheap to detect. 6) Easy and fast assay. 7) High reproducibility and 8) Easy exchange of data between laboratories. The DNA extraction method includes separation of DNA from obviously occurring plant cell ingredients such as polysaccharide and polyphenolic compounds (Pandey *et al.,* 1996; Porebski *et al.,* 1997) tracked by deproteinisation of the aqueous solution containing the

DNA, precipitation and purification of DNA. The difficulty often met during the extraction is that these compounds form multiplexes with and become bound to nucleic acids (Varadarajan and Prakash, 1991). Polysaccharides are visually apparent in DNA extractions by their sticky, glue-like texture and make the DNA uncontrollable in pipetting and unamplifiable in PCR by inhibiting Taq polymerase (Fang *et al.*, 1992). Finally, during translation, the RNA sequence is translated into a sequence of amino acids as the protein is formed (Alberts *et al.*, 1983).

2.3.1.2 The Polymerase Chain Reaction (PCR).

DNA polymerase only acts from 5' to 3'. Since one strand of the double helix is 5' to 3' and the other one is 3' to 5', DNA polymerase synthesizes a second copy of the 5' to 3' strand (the lagging strand), (Ogawa and Okazaki, 1980). These steps of template denaturation, primer annealing and primer extension comprise a single "cycle" in the PCR amplification methodology (Andy Vierstraete, 2001). According to Sambrook *et al.*, (1989), to obtain the denaturation of DNA, the temperature is usually increased to 93 - 96°C, annealing or rehybridisation at lower temperature (usually 55 - 65°C) and primer extension at 72°C which is the ideal working temperature for the *Taq* DNA polymerase. The first thermostable DNA polymerase used was the *Taq* DNA polymerase which was isolated from the bacterium *Thermus aquaticus* (Saiki *et al.*, 1988). Newton and Graham (1994), characterized some thermostable DNA polymerases that are currently in use for PCR.

2.3.1.3 Molecular markers and application in diversity studies

Molecular markers are a definite means to collect desirable agronomic traits since they are based on the plant genotypes and also independent of environmental variation (Franco *et al.*, 2001). Scientists have considered genetic variation in tomato cultivar groups using numerous molecular techniques including Amplified Fragment Length Polymorphism (AFLP), Restriction Fragment Length Polymorphism (RFLP), Simple Sequence Repeats (SSR) and

Random Amplified Polymorphic DNA (RAPD) (Bredemeijer *et al.,* 1998, Villand *et al.,* 1998, Park *et al.,* 2004 and Garcia-Martinez *et al.,* 2006). According to Dongre and Parkhi (2005), RAPD is the first PCR-based molecular marker technique to develop DNA marker for the detection and monitoring of pedigree breeding record of inbred parents and to determine genetic relations amongst genotypes. It is an effective method for varietal documentation, study of polymorphism, gene mapping, biodiversity, genetic map construction, hybridization and phylogenetic relationship in tomato varieties (Sharma and Sharma, 1999).

2.3.1.4 Random amplified polymorphic DNA (RAPD).

The PCR-based RAPD technique (Williams *et al.,* 1990) corresponds with straight DNA fingerprinting. RAPD analysis is theoretically simple. This protocol differs from the normal PCR (Erlich 1989) in that only a single random oligonucleotide primer is employed and no previous knowledge of the genome is necessary. The amplification protocols are resolved on agarose gels and polymorphisms serve as dominant genetic markers which are inherited in a Mendelian fashion (Williams *et al.* 1990; Carlson *et al.* 1991; Martin *et al.,* 1991; and Welsh, Peterson and McClelland 1991). According to Caetano-Anolles *et al*. (1991), two methods of identifying RAPD markers have been described. These include DNA Amplification Fingerprinting (DAF) and Arbitrarily Primed Polymerase Chain Reaction (AP-PCR). DAF uses short random primers of 5-8 bp and comparable to the greater number of amplification products by polyacrylamide gel electrophoresis and silver staining. AP-PCR uses slightly longer primers (such as universal M13) and amplification protocol are radioactively labeled and also determined by polyacrylamide gel electrophoresis (Welsh and McClelland 1990; Welsh *et al.,* 1991b). Normal RAPD analysis is performed according to the original methods (Williams *et al.,* 1990) using short oligonucleotide primers of random sequence which are commercially available (Operon Technologies, Inc., Alameda, Calif.). RAPD markers have

been used in demonstrating the introgression of two maternal genomes in an iris hybrid species (Arnold *et al.,* 1991). According to Williams *et al.* (1990), nearly all RAPD markers are dominant, and therefore, it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous or homozygous. Codominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely.

2.3.1.5 Simple Sequence Repeats (SSR)

The term microsatellites, invented by Litt & Lutty (1989) and also known as Simple Sequence Repeats (SSR), are sections of DNA that consist of tandemly repeating mono-, di-, tri-, tetra- or penta-nucleotide units. These are arranged throughout the genomes of most eukaryotic species (Powell *et al.,* 1996). SSRs are short cycle repeats with 1 to 10 bp, most classically, 2-3 bp. They are extremely variable and consistently circulated throughout the genome (Hajeer *et al.,* 2000). SSR polymorphisms can be visualised by agarose or polyacrylamide gel electrophoresis (Dean *et al.,* 1999). Microsatellite alleles are one of alternative forms of a gene that can exist at a single locus which can be noticed, using numerous methods: ethidium bromide, silver staining, radioisotopes or fluorescence. These markers are simply automated, highly polymorphic, and have good analytical determination, thus making them a preferred choice of markers (Matsuoka *et al.,* 2002).

2.3.1.6 Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism is a technique that exploits variations in homologous DNA sequences (Vos *et al.,* 1995). It refers to an alteration between samples of homologous DNA molecules that come from differing locations of restriction enzyme sites, and to a related laboratory technique by which these segments can be illustrated. RFLP procedure is intended as a complex method, which allows high numbers of polymorphic

genetic markers to be identified, independent of environmental influences and tissue type (Vos *et al.,* 1995). The simultaneous detection of 10-40 unlinked and highly polymorphic loci provides a whole genome "fingerprint" pattern which is expected to show differences between any two unrelated individuals (Jeffreys *et al.,* 1985). An important variation on two-dimensional RFLP analysis has been developed (Hayashizaki *et al* 1994). They reported the detection of several thousand spots on two-dimensional gels derived from individual mice (Hatada *et al.,* 1991). RFLP analysis ideally results in a series of bands on a gel, which can then be scored either for present or absent of particular bands, or as codominant markers (IPGRI and Cornell University, 2003). RFLP are codominantly inherited and, as such, can estimate heterozygosity and required large amounts of DNA (IPGRI and Cornell University, 2003)

2.3.1.7 Single Nucleotide Polymorphism (SNP)

A single-nucleotide polymorphism is a DNA sequence variation occurring when a single nucleotide A, T, C or G in the genome differs between members of a biological species or paired chromosomes in a human (Barreiro *et al.,* 2008). The genomic distribution of SNPs is not homogenous; SNPs usually occur in non-coding regions more frequently than in coding regions or, in general, where natural selection is acting on the allele of the SNP that constitutes the most favorable genetic adaptation (Barreiro *et al.,* 2008). Genetic recombination can also determine SNP density (Nachman, 2001), which can also be predicted by the presence of microsatellites. SNPs represent the most frequent form of polymorphism in the human genome (Wang *et al.,* 1998). In multiple-gene surveys, estimates of nucleotide diversity in the human genome range between 3.7×10^{-4} and 8.3×10^{-4} differences per base pair (Wang *et al.,* 1998; Cambien *et al.,* 1999; Cargill *et al.,* 1999; Halushka *et al.,* 1999; Sachidanandam *et al.,* 2001; Stephens *et al.,* 2001*a*). From these and other studies of nucleotide diversity, it has been estimated that a common SNP occurs once every ~600 bp

(Kruglyak, 2001). Assuming that the average gene in the human genome spans ~27 kb (Lander *et al.,* 2001; Venter *et al.,* 2001), ~50 common polymorphisms may be existing in such a gene. Though the number of common variants per gene is limited, the quantity of current genotyping technologies is insufficient for genotyping all existing common variants in all but the smallest of genes (Nickerson *et al.,* 2000).

2.4 Measurement of intra population genetic diversity

Intra population genetic diversity is the concept used to measure the degree of polymorphism within a population, Nei *et al.* (1979), and one commonly used measure of genetic diversity first introduced by Nei *et al.* (1979). This measure is also defined as the average number of genetic differences per site between any two DNA sequences chosen randomly from the sample population, which may be used to monitor diversity within or between ecological populations, to examine the genetic variation in crops and related species, (Kilian *et al.,* 2007) or to determine evolutionary relationships, (Yu, *et al.,* 2004). Intra population genetic diversity can be calculated by examining the DNA sequences directly, or may be estimated from molecular marker data, such as Random Amplified Polymorphic DNA (RAPD) data (Borowsky *et al.,* 2001) and Amplified Fragment Length Polymorphism (AFLP) data (Innan *et al.,* 1999).

2.4.1 Frequency of variants

Frequency of variants is the percentage of all alleles at a given locus in a population gene pool represented by a particular allele (King *et al.,* 2006). Low frequency variants are enriched for functional variants, particularly for nucleotide changes that affect protein function, and are therefore putatively more related to disease (Maxwell *et al.,* 2010, Kenny *et al.,* 2012). Additionally, most rare variants are isolated or show very little distribution among

areas (Jean. 2012; Del la Vega, 2011). This may be particularly important in terms of genetic fitness, since rare variants are enriched for deleterious alleles.

2.4.1.1 Effective number of alleles (Ae)

Kimura and Crow's estimation of the effective number of alleles *(*Ne) in a population as 1 + 4 *N,U* (Kimura and Crow 1964) has formed the basis of a large body of theoretical work in population genetics. Ohta and Kimura (1973a, 1973b) presented a new model for the estimation of the effective number of alleles in populations. They indicated that inspection of electrophoretic data on allozyme variation may reveal only a fraction of the total number of amino acid changes, as oppositely charged amino acid alterations cancel in their effect upon electrophoretic mobility. To compensate for this, a new estimator of Ne, -I- 8 NeU, was proposed. The new estimator of *n,* proposed by Ohta and Kimura is based upon a set of rather unrealistic biochemical assumptions. Because of this, the suitability of employing the new formulation to analyze experimental data needs to be questioned. Ohta and Kimura's new model of *Ne* is founded upon the conception that electrophoretically detectable alleles are able to "mutate only to one of the two adjacent (electrophoretic) states. One positive and one negative change in charge cancel each other, leading the allele back to the original state" rather than producing a third discrete state as assumed in the previous model of Kimura and Crow (1964). Therefore, the conception of protein structure essential to Ohta and Kimura's new design of Ne, that "one positive and one negative change in charge cancel each other, leading the allele back to the original state", seems a limited one. It signifies only one alternative among a range of biochemical possibilities. Really, the two designs of Ne, 1 + 4 *N,U* and -t 8 *N,U,* define the limiting cases of the spectrum of non-identification possibilities among electrophoretic alleles: in the previous case, no opposite charges cancel, and all electrophoretic alleles are recognized, while in the latter case all opposite charges cancel, and a minimum number of electrophoretic alleles are recognized.

2.4.1.2 Average expected Heterozygosity (He) {(Nei's genetic diversity, Dj)}

According to, Kotzé and Muller, (1994), heterozygosity is a measure of genetic variation within a population. A high level of average heterozygosity at a locus could be expected to correlate with high levels of genetic variation at loci with critical importance for adaptive response to environmental changes (Kotzé and Muller, 1994). The expected heterozygosity (also called gene diversity) is calculated from individual allele frequencies (Nei, 1987). The FSTAT (Goudet, 1995), GENETIX (Belkhir *et al.,* 1996-2004), R-package, Microsatellite Analyzer (Dieringer and Schlštterer, 2003) and MSTollkit (Park, 2004) computer programs can be used to estimate both observed and expected heterozygosity per locus and population and across all populations analysed. Reading the sampling variance of heterozygosity and genetic distance, Nei and Roychoudhury (1974) decided that for approximating average heterozygosity and genetic distance a large number of loci rather than a large number of individuals per locus should be used when the total number of genes to be observed is fixed. They also recognized Nei and Roychoudhury (1974) theoretical conclusion that a relatively dependable estimate of average heterozygosity can be obtained from a small number of individuals if a large number of loci are examined.

2.4.1.3 Polymorphism or rate of polymorphism (Pj).

Bodmer *et al.,* (1981) defined gene as polymorphic if the frequency of one of its alleles is less than or equal to 0.95 or 0.99

$Pj = q \leq 0.95$ or $Pj = q \leq 0.99$

Where,

Pj = rate of polymorphism

q = allele frequency

They also defined rare alleles as those with frequencies of less than 0.005. The limit of allele frequency, which is set at 0.95 (or 0.99), is random, its objective being to help identify those genes in which allelic variation is common (Bodmer el at. 1981).

2.4.1.4 Proportion of polymorphic loci.

Polymorphism occurs when two or more clearly different phenotypes exist in the same population of a species or the existence of more than one form or morph. In order to be classified as such, morphs must occupy the same habitat at the same time and belong to a panmictic population (one with random mating) (Ford 1965). Hudson and Kaplan (1995) and Enrique *et al.,* (1996) derived terminologies for predicting the nucleotide diversity at neutral loci under the related selection ideal (Charlesworth *et al.*,1993) which is based on the constant appearance of linked deleterious mutations in the population. Barton (1995) made similar sources for the complex probability of a favorable allele. Robertson (1961) and Santiago and Caballero (1995) basically showed that the effective size of a population is a function of the variance of the cumulative selective values associated with neutral genes. Wiehe and Stephan (1993) finally show that the two categories of estimators of polymorphism essentially mean heterozygosity per site and proportion of segregating sites are related to size. Calculations can still be made from effective size theory.

2.4.1.5 Richness of allelic variants (A).

The concept of bottlenecks in the effective population size predicts a strong decrease of allelic richness and a more limited decrease of *H* at neutral loci since rare alleles will be more readily affected by drift than the more frequent ones (Nei *et al., 1*975). Wade and McCauley (1988) proposed that a decrease in *H* and an increase in levels of variation were to be likely in recently founded populations, but Slatkin (1977) claimed that colonization could institute a form of gene flow, when the number of organizers is large enough. Cornuet and Luikart

(1996) and Nei *et al., (*1975) state that population bottlenecks may initially reduce levels of genetic diversity and admixture effects which could take place when immigrating populations encounter. This might result in a secondary increase of diversity. Numerous pragmatic inquiries reflect only *H*, the possibility that two alleles sampled at random are different. This parameter is called *gene diversity* by Nei (1973). This method is preventive, since allelic richness has quite diverse dynamics and may be more useful in recognizing historical processes such as bottlenecks (Luikart *et al.,* 1998) and population admixture (Chakraborty *et al.,* 1988).

2.4.1.6 Average number of alleles per locus.

An allele is one of a number of alternative forms of the same gene or same genetic locus (Collins *et al.,* 2010). It is the alternative form of a gene for a character that produces different effects. Sometimes different alleles can result in different observable phenotypic traits, such as different pigmentation. Yet, many genetic variations result in little or no observable variation. Loci with more alleles are generally thought to produce more precise estimates of genetic distances than loci with few alleles, especially for closely related populations. However, loci with a large number of alleles can be difficult to score and take up more space on electrophoretic gels (Nei *et al*., 1983).The average number of alleles per locus is the sum of all detected alleles in all loci, divided by the total number of loci

$$N = (1/K) \sum_{I-1}^{K} ni$$

Where,

K = the number of loci

ni = the number of alleles detected per locus

This measure provides complementary information to that of polymorphism. It requires only the counting of the number of alleles per locus and then calculating the average. It is best

applied with codominant markers, because dominant markers do not permit the detection of all alleles.

2.5 Methods of displaying relationships

In order to cluster the items in a data set, some means of quantifying the degree of association between them is required. This may be a distance measure or a measure of similarity or dissimilarity. There are a number of similarity measures available but the choice of similarity measure can have an effect on the clustering results obtained (Willett. 1988). According to Willett (1983), similarity coefficients in cluster-based recovery suggest that it is important to use a measure that is regularized by the length of the document vectors. A diversity of distance and similarity measures is given by Anderberg (1973) while those most suitable for comparing document vectors are discussed by Salton (1989). The Dice, Jaccard and cosine coefficients have the attractions of simplicity and regulation and have often been used for document clustering. Moreover, many clustering methods are based on the coupling of the most similar documents or clusters, so that the similarity between every pair of points must be known. (Willett 1980; Perry and Willett 1983).

2.5.1 Clustering method.

The method of clustering is to reduce the amount of data by categorizing or grouping similar data items together. One of the motivations for using clustering method is to provide automated tools to help in constructing categories or taxonomies (Jardine and Sibson, 1971; Sneath and Sokal, 1973). Clustering methods can be divided into two basic types: hierarchical and partitional clustering. (Anderberg, 1973, Hartigan, 1975, Jain and Dubes, 1988, Jardine and Sibson, 1971, Sneath and Sokal, 1973, Tryon and Bailey, 1973). Hierarchical clustering proceeds sequentially by either merging smaller clusters into larger

ones or by splitting larger clusters. Partitional clustering, on the other hand, attempts to directly decompose the data set into a set of disjoint clusters.

2.5.1.1 Hierachical clustering.

Hierarchical clustering is one of the most regularly used methods in unconfirmed learning. Given a set of data points, the output is a binary tree (dendrogram) whose leaves are the data points and whose internal nodes characterize nested clusters of various sizes. The tree arranges these clusters hierarchically, where the hope is that this hierarchy agrees with the spontaneous organization of real-world data (Jain and Dubes, 1988). The method for hierarchically clustering data is a bottom- up agglomerative procedure (Duda & Hart, 1973). It starts with each data point assigned to its own cluster and iteratively merges the two closest clusters together until all the data belongs to a single cluster. The cluster structure resultant from a hierarchical agglomerative clustering method is often displayed as a *dendrogram*, (Willett, 1988). The most commonly used hierarchical agglomerative clustering methods and their characteristics are: the single link method which joins, at each step, the most similar pair of objects that are not yet in the same cluster, (Jardine and Sibson 1971). The group average link method uses the average values of the pairwise links within a cluster to determine similarity (Lorr. 1983).

2.5.1.2 Objectives for clustering.

The objective of clustering is descriptive, that of classification is predictive (Veyssieres and Plant, 1998). Meanwhile the objective of clustering is to discover a new set of categories; the new groups are of interest in themselves and their valuation is inherent. In classification tasks, however, an important part of the valuation is extrinsic. Meanwhile, the groups must reflect some reference set of classes. (Tryon and Bailey, 1970).

2.5.1.3 Types of data for clustering.

a. Binary data – SMC/Jaccard's coefficient.

In the case of binary traits, the distance between objects may be calculated based on a contingency table. A binary trait is symmetric if both of its states are equally valued. In that case, using the simple matching coefficient can assess dissimilarity between two objects:

$$d(xi; xj) = \frac{r + s}{q + r + s + t}$$

Where q is the number of attributes that equal 1 for both objects;

t is the number of attributes that equal 0 for both objects; and

s and r are the number of attributes that are unequal for both objects.

A binary attribute is asymmetric if its states are not equally important (usually the positive outcome is considered more important). In this case, the denominator ignores the unimportant negative matches (t). This is called the Jaccard coefficient:

$$d(xi; xj) = \frac{r}{r + s + t}$$

b. Qualitative data

These refer to characters or qualities, and are either binary or categorical:

Binary, taking only two values: present (1) or absent (0)

Categorical, taking a value among many possibilities, and are either ordinal or nominal:

Ordinal: categories that have an order

Nominal: categories that are unrelated

c. Quantitative data.

These are numerical and are either continuous or discrete:

Continuous, taking a value within a given range

Discrete, taking whole or decimal numbers

(IPGRI and Cornell University, 2003)

CHAPTER THREE

3.0 Materials and Methods

Two experiments were carried out. The first one was a field experiment which was carried out in two locations (CRI, Kwadaso and KNUST). The second experiment was a laboratory experiment which was carried out at the Biotechnology laboratory of KNUST.

3.1 Field experiment

3.1.1 Experimental site

The experiment was conducted at the Department of Horticulture experimental field, KNUST, and Horticulture Division of CSIR – CRI, Kwadaso. Total rainfall and mean sunshine recorded for KNUST, during the experiment was 850.5 mm and 30.4 hours respectively. Minimum and maximum mean temperature were however 21.9˚C and 31.1˚C respectively. The experimental area lies between latitude 06, 43˚ North and longitude 001, 36˚ West. Similarly, the total rainfall and mean sunshine recorded at Kwadaso was 531.1 mm and 32.4 hours respectively. Temperatures of 24.1˚C and 32.2˚C were recorded as minimum and maximum temperatures. Kwadaso station lies between latitude 06, 42˚ North and longitude 001, 4˚ West.

3.1.2 Experimental design

Four tomato varieties from the USA and a local check from CSIR-Crops Research Institute (CRI), Kumasi, Ghana were planted in the fields at the Department of Horticulture, KNUST and, Horticulture Division of the CSIR-CRI, Kwadaso. The size of the plots (KNUST and CSIR-CRI) was 264 m^2 (12 m x 22 m). The trial was laid out in a randomized complete block design with three replicates at both locations. Each replicate contained five varieties as treatments, Heinz, Shasta, Op-B149, Op-B155 and CRI-P00. Each plot had double rows and

plants spaced at 100 cm x 50 cm with a plant population of 24 per plot. Seeds for the experiment were nursed on June 21, 2013 and transplanted three weeks thereafter. YaraMila Winner (150 kg per hectare) and YaraLiva Nitrabor (50 kg per hectare) were used as basal fertilizer at transplanting, respectively. Staking of plants was done accordingly when the plants were three weeks old after transplanting to permit easier observation of plant characteristics. Other standard agronomic practices such as watering and weeding were done. Spraying of fungicides which includes Victory 50 g plus 15 liters water, and alternated with 50 g Triamagol plus 15 liters water were done after one week at vegetative stage. 300 ml TopCop plus 15 liters water was also alternated with 60 g Funguran plus 15 liters water after one week at the flowering stage. The insecticides were used as follow: 20 g Golan at vegetative stage, 50 ml Deltapaz + 35 ml Rim-On and alternated with Deltapaz with 50 ml Karate + Rim-On after one week at flowering stage and at fruiting stage 50 ml of Karate.

3.1.3 Data Collection

Data were taken as soon as plants were established on the fields. Data included number of days to first, 50 % and 100 % flowering. The rest included plant height (cm), stem length (cm), stem diameter (mm), number of leaves under the first inflorescence (3-few and 7-many), number of flowers per inflorescences, number of plant harvested, marketable and non-marketable fruits, average weight of marketable fruits, total marketable fruit weight, yields (t/ha), fruit shape (5-heart shape, 4-high rounded, 3-rounded and 2-slightly flattened), plant size (5-intermediate and 7-large), growth habit (2-determinate and 3- semi-determinate), disease and insect pest incidence, post-harvest characters such as brix and shelf life including other sensory characters. Harvesting were done six times. A refractometer was used to measure the brix (solid soluble). Data were then subjected to Analysis of Variance (ANOVA) using the Genstat (12th edition) Statistical package. LSD at 5% was used to separate the treatment means.

3.1.4 Sensory Evaluation

A total of twenty (ten males and ten females) volunteers participated in a sensory evaluation test at CRI, Kwadaso. They included staffs of CSIR-CRI (Research Scientists, Technicians and Administrative assistants). Selected tomato fruits from each variety was used to prepare soup, stew, and salad. The five tomato accessions were labelled as 100, 200, 300, 400, and 500 without displaying the actual variety names to the volunteers or participants. Sensory evaluation forms were then given to the participants to score for taste, flavour, sweetness, appearance, colour, and mouth-feel. They were also asked to rank the attributes mentioned above.

Data was then subjected to Analysis of Variance (ANOVA) using the Genstat (12th edition) Statistical package. LSD at 5% was used to separate the treatment means.

3.2 Laboratory Experiment

Following field evaluation of the accessions, the genetic diversity of the five (5) varieties of tomato accessions were again examined at the DNA level using SSR primers designed for tomato DNA fingerprinting. This was carried out to confirm the diversity revealed by morphological markers.

3.2.1 The study area

The ex-situ characterization was carried out under controlled conditions at Biotechnology Laboratory at the Department of Crops and Soil Sciences, KNUST.

3.2.2 DNA extraction and purification

Total genomic DNA was extracted from young freshly harvested leaves of five (5) tomato accessions from the experimental fields of the Department of Horticulture, KNUST, Ghana

using a modified DNA isolation method described by Takrama, 2000 CRIG. This protocol consisted of cell lysis, DNA extraction and DNA precipitation and purification.

Twenty milligram (20 mg) of tender varieties of tomato leaves of each accessions were weighed into 2 ml eppendorf tube, ground to fine powder in liquid nitrogen using Teflon pestle. To each tube, 800 µl of lysis buffer containing 2 g CTAB, 2 g PVP, 28 ml NaCl, 4 ml EDTA (pH 8.0), 10 ml Tris – HCl (pH 8.0), and 0.1 ml 2 –mercaptoethanol was added and shaken several times until a homogenous mixture was obtained so as to lyse nuclear membranes. The mixture was incubated at 65^0C in a shaking water bath for 10 minutes and the tube shaken by hand two times at five (5) minutes intervals to ensure uniform temperature within the tube.

The protein contaminants from the cell lysate were removed by adding equal volume (800 µl of chloroform isomyl-alcohol and mixed gently by inversion of the tube. The incubated samples were immediately placed on ice for 30 minutes. The samples were centrifuged at 14000 rpm for 5 minutes using micro centrifuge and the upper DNA containing phase transferred into fresh tubes without disturbing the tube.

An equal volume of ice cold isopropanol, which had been stored at -20^0C, were added to each tube with the DNA containing supernatant and the tube gently inverted a few times to precipitate the DNA. The samples were incubated at -20^0C for 8 hours. The samples were centrifuged again at 14000 rpm for 5 minutes, the supernatant was discarded and the DNA pellet washed with 500 µl of 80% ethanol. The samples were centrifuged at 6000 rpm for 4 minutes. DNA pellets were air-dried at room temperature on the laboratory bench for 10 minutes to remove the remaining ethanol droplets from the tube and redissolved in 50 µl of 1X TE (Tris and EDTA) buffer. 1µl of RNase A (6mg/ml) was added to the redissolved DNA

samples and incubated at 37^{0}C for 10 minutes to remove any RNA remaining. The DNA was purified with 400 µl of 6M bromophenol blue, incubated on ice for 30 minutes and then centrifuged at 14000 rpm for 5 minutes. The supernatant was transferred into a clean 1.5 ml eppendorf tube and 14000 rpm for 15 minutes. The DNA pellets were washed with 400 µl of 80% ethanol, air-dried at room temperature and redissolved in 50 µl 1X TE (Tris-ethylenediaminetetracetic acid) buffer and centrifuged at high speed for 30 seconds to remove all insolubles.

3.2.3 DNA quality testing and quantification.

The DNA quality and quantity were determined using a spectrophotometer (Biochrom Libra S12) and taking the absorbance reading at 260 nm and 280 nm (A_{160} and A_{280} respectively) levels. The stored DNA samples were thawed, mixed thoroughly on a GENIE Vortex-2 (Scientific Industries, UK) and 5 µl of genomic DNA added to 495 µl of 1X TE (Tris-EDTA buffer) in a 1 ml microcentrifuge tube and mixed well before reading the absorbance at 260 nm and 280 nm wavelengths. The 1X TE (Tris-EDTA buffer) was used as a reference sample to set the spectrophotometer at 260 nm and 280 nm wavelength (blanking). The diluted DNA sample was loaded to the cuvette of the spectrophotometer for estimation of the absorbance. The quality of DNA was assessed using the absorbance ratio at 269 to that at 280 wavelengths (A260/A280). If this ratio is 1.8-2.0, the absorption is probably due to nuclei acids, hence it is of good quality. A ratio of less than 1.8 indicates there may be proteins and/or other UV absorbers in the sample. However, a ratio higher than 2.0 indicates the samples may be contaminated with RNA or phenols (CIMMYT, 2005).

DNA quantity was calculated according to Weising *et al.*, (2005) as

DNA (µg/µl) = A_{260} x 50

Where, A_{260} is the absorbance at 260 nm.

Thus the concentration of DNA in µg/ml was calculated as:

DNA (µg/ml) = [A_{260} x 50] x DF where DF is the dilution factor.

From the quantities of DNA calculated, the appropriate volumes were pipette into samples tubes and topped up with sterile distilled water (SDW) to make concentration of 10ng/µl used for polymerase chain reaction amplifications. The integrity of the DNA was also assessed by running the DNA samples on 2% agarose gel (0.1% ethidium bromide). Each well contained a mixture of 2 µl of loading and 10µl of the genomic DNA sample. The gels were run with 1X TAE buffer from the cathode to the anode with a constant voltage of 120V for 45 minutes. They were visualized after electrophoresis with a UV transilluminator (UVP Inc., USA) and photographed with a canon digital (Canon, Power Shoot A4000 IS 16MP) camera. High quality DNA samples usually appear as thick bands. For samples with very weak concentration, no further dilution was done. DNA samples with no visible shearing were then selected for subsequent PCR amplification.

3.2.4 Test run for SSR primers

Polymerase chain reaction (PCR) optimization was performed for all the SSR markers and best performing conditions identified. A fraction of the total number of accessions was used for the polymerase chain reaction (PCR) during the primer testing. A total of 15 SSR primer pairs were used for the study (Table 1).

3.2.5 Molecular markers and polymerase chain reactions.

Fifteen highly polymorphic SSR markers (Table 1), obtained from Metabion International Laboratory, Germany which are widely distributed in the tomato genome were used in genotyping the accessions. Amplifications were carried out in Techne prime thermal cycler (Labnet International Inc., California, USA) and GeneAmp PCR System 9700 (Applied Biosystems, USA) of 96-well plates with heated lid to reduce evaporation. The DNA from 5

accessions were fingerprinted using SSR markers in a 10 µl reaction volume of master mix containing, 1.5 mM of 5X Buffer A with $MgCl_2$, 10 mM of dNTPs (Deoxynucleotide Triphosphates), 0.3 µM each of forward and reverse primer and 0.08 µl *Taq* polymerase. Water was added to make the final volume. Reactions were conducted at an initial denaturation step at 94^{0}C for 5 min; a touchdown procedure of 94^{0}C for 30 secs denaturation, annealing step of 65^{0}C for 20 secs reducing at -1^{0}C per cycle for 10 times annealing temperature depending on marker), followed by 94^{0}C for 30 secs, 55^{0}C for 30 secs; 72^{0}C for 1 min for 42 cycles and a final extension/elongation step at 72^{0}C for 10 min and then held at 4^{0}C. The amplified products were stored at -20^{0}C until required to run gels.

3.2.6 Agarose gel electrophoresis (AGE).

The PCR products were separated using horizontal Agarose gel electrophoresis (AGE). The amplified DNA fragments were separated on 2% Agarose gel at 120v for 45 minutes – 1 hour in TBE (Tris-borateetheldiamineteraacetic acid) (1X) using a cell electrophoretic apparatus (MS Major Science, UK) and BIO RAD (Criterion TM cassettes). 1X DNA loading dye was added to the PCR products for visual tracking of DNA migration during electrophoresis. A 100bp DNA marker (gene rule) was used as a reference to estimate the size of specific DNA bands in the PCR amplified products. After the AGE, the DNA fragments were visualized in the gels by staining with 0.1% ethidium bromide for 3 min and photo-documented with a digital camera under UV light. Banding patterns were then visualized and compared between individuals. The gel stored in distilled water. PCR products scored for present (1) or absent (0) of bands.

Table 3.1: Set of tomato microsatellite markers used in DNA fingerprinting.

S/NO	Marker name	Primer sequence (5′ - 3′)	No. of Bases
1	TGS0001F	GCGACCCTCTATTGAACTTGAAGAC (F)	25
		ACAAATCAAAGGAACAATTTCAA (R)	23
2	TGS0002F	GCAAACGTGTTCGAGTTCGTG (F)	21
		CCACACAATAAAGACAGAAAAATG (R)	24
3	TGS0003F	ATGCATGCGTGTGTGTTGTA (F)	20
		GTGTGTGTGTGTGTGTGTGTGT (R)	22
4	TGS0004F	GCAATTTATTTTCATTTGTTATACCGGA (F)	28
		ACCGAGACTCCTGGCTCATA (R)	20
5	TGS0005F	GACAAAAATTTTCCACACGGC (F)	21
		TCTCTTATAATTTTGTTGAGTCTCTGA (R)	27
6	TGS0006F	GTCGCATAAATATGGACAACGA (F)	22
		TTTTTAAAATACCATTCCAGAAAAA (R)	25
7	TGS0007F	GTGGATTCACTTACCGTTACAAGTT (F)	25
		CATTCGTGGCATGAGATCAA (R)	20
8	TGS0008F	GCGGTGTGAAATACAACAAGACG (F)	23
		CTCGACAAGCTAATTTCTGGG (R)	21
9	TGS0009F	GCGAAGCAAAAGAAAATTGGG (F)	21
		CACCACGAAGGCTGTTGTTA (R)	20
10	TGS0010F	TTGAAAAGCTGAAAAGTCAATCA (F)	23
		GAGAGGTGCCACATCACCTT (R)	20
11	TGS0012F	GTCCCTACCCCACAAATTGAA (F)	21
		AGGTACAACTCACCTCCCCC (R)	20
12	TGS0013F	GGTGGACATATGAGAAGACCTTG (F)	23
		TCATTTTCCAATGGTGTCAAA (R)	21
13	TGS0014F	GTGAAGACGAAAAACAAGACGA (F)	22
		CCTTCCCCTTTTGTCTCTCC (R)	20
14	TGS0020F	TCTTTCAACTTCTCAACTTTGGC (F)	23
		GCCGACTTCAAAAACTGCTC (R)	20
15	TGS0023F	GTCCAAATTAAAAACTAACCGCA (F)	23
		TTTCCAAAATGACCTAGCGG (R)	20

NB: F: Forward primer, R: reverse primer

3.2.7 Gel scoring of DNA fragments.

After staining the gels with ethidium bromide, size matching/calling was done using a reference standard of KAPA Universal DNA Ladder Kit 100 bp ladder DNA marker which ranges from 100 bp – 10000 bp. The bands on the gel were scored for present (1) or absent (0) of bands together with their respective sizes. For each marker, alleles for the data set were scored according to size of base pairs of the 100 bp ladder DNA marker. This procedure was

conducted for each marker until all alleles were scored with the smallest and largest sized alleles representing the start of the first scoring and the end of the last scoring, respectively.

3.2.8 Data analysis.

The molecular data were subjected to gene diversity and genetic differentiation analysis. To determine the relationship among accessions based on hierarchical cluster analysis, the individual alleles were scored for each genotype across all SSR markers used for the study. The data in this form were used to calculate genetic distances between pairs of tomato accessions from the comparison of the band scores. Pairwise distance matrices were computed using the NTSYSpc 2.20 software (Jaccard's dis/similarity coefficient) to generate structure dendrogram. Power Marker computed programme, version 3.25, was also used in conducting allelic frequency analysis. To estimate genetic diversity among the accessions, SSR loci were recorded diploids with single bands taken to indicate the presence of two identical alleles. Genetic diversity was estimated using five statistics averaged over loci, polymorphic information content (PIC), mean number of alleles per locus or allelic richness (A), the average observed heterozygosity (H_o), and the average gene diversity (H_e) were computed, according to Nei (1983).

CHAPTER FOUR

4.0 RESULTS

4.1 Agronomic characteristics of five tomato varieties cultivated at KNUST, Kumasi.

The agronomic characteristics of five tomato varieties studied are summarized in Table 4.1. The tomato varieties varied significantly in almost all the agronomic traits. Major differences were found among the following genotypes; Shasta and Heinz, Shasta and OP-B149, CRI-P00 and Heinz, CRI P00 and OP-B149 based on the number of plants per plot. Shasta gave the highest plant population per plot with Heinz and OP-B149 recording the least plant population per plot. On the number of days to first flowering, 50 % flowering and 100% flowering, CRI-P00 recorded the least number of days whereas OP-B155 took the highest number of days to first flowering, 50% flowering and 100 % flowering. Significant differences were also found between OP-B155 and Shasta such that Shasta also recorded less number of days to 1st flowering. Based on the number of days to 50 % flowering significant differences were also found between CRI-P00 and OP-B149 with CRI-P00 still recording the least number of days. For number of days to 100 % flowering, however, CRI-P00, Heinz and Shasta, all recorded less number of days as compared to OP-B149 and OP-B155. The tallest average plant height (cm) was recorded by Shasta (98.2 cm), while OP-B155 produced the shortest average plant height (81.1 cm). Heinz (14.9 cm) and Op-B149 (9.7 cm) gave the longest and shortest stem length respectively. The biggest and smallest stem diameter were found on OP-B155 and Shasta, respectively.

Table 4.1: Agronomic characteristics of five tomato varieties at KNUST, Kumasi

Genotypes/ accessions	Plants/plot	No. of days to 1st flowering	No. of days to 50% flowering	No. of days to 100% flowering	Plant Height (cm)	Stem length (cm)	Stem diameter (mm)
HEINZ	21.0	26.3	29.3	32.0	85.4	14.9	14.2
SHASTA	23.0	26.0	29.7	33.0	98.2	13.7	13.5
CRI-POO	23.7	24.3	27.0	31.7	90.2	13.2	13.8
OP-B149	21.0	27.0	30.7	38.0	83.9	9.7	15.2
OP-B155	22.7	29.0	30.7	38.0	81.1	10.7	16.2
MEAN	22.3	26.5	9.5	34.5	87.8	12.4	14.5
LSD (0.05)	1.7	2.7	1.4	2.5	14.9	1.5	3.6
CV (%)	4.1	5.5	2.4	3.8	9.0	6.3	13.1

4.2 Agronomic characteristics of five tomato varieties cultivated at CRI, Kwadaso

Table 4.2 summarises the agronomic characteristics of the five tomato varieties studied at Kwadaso.. The tomato varieties varied significantly in almost all the agronomic traits. Significant difference was found between Shasta and OP-B149 on the plant population per plot. Furthermore there were also some significant differences between Shasta and OP-B155 on the plant population per plot. On the number of days to first flowering, 50% flowering and 100% flowering, CRI-P00 recorded the least number of days whereas OP-B155 took highest number of days to first flowering, 50% flowering and 100% flowering. Significant differences were however, found between OP-B155 and CRI-P00, OP-B155 and Shasta based on the number of days to first flowering. Furthermore, there were significant differences on the number of days to first flowering between OP-B155 and Heinz, OP-B149 and CRI-P00. Also, significant differences were found between OP-B149 and CRI-P00, OP-B155 and CRI-P00 based on the number of days to 50% flowering. OP-B149 and CRI-P00, OP-B149 and Shasta, OP-B149 and Heinz, OP-B155 and CRI-P00, OP-B155 and Shasta,

OP-B155 and Heinz showed significant differences based on the number of days to 100% flowering.

The utmost average plant height (cm) was recorded on Shasta (84.1 cm). OP-B155 however, gave the lowest average plant height (57.6 cm). The trial however, gave significant differences on all the varieties in terms of average plant height. Shasta (16.9 cm) and OP-B155 (10.5 cm) gave the longest and shortest stem length respectively. The widest and lowest stem diameter were found on OP-B149 and Heinz respectively (Table 4.2).

Table 4.2: Agronomic characteristics of five tomato varieties at CRI-KWADASO, Kumasi

Genotypes/ accessions	Plants/ plot	No. of days to 1st to flowering	No. of days to 50% flowering	No. of days to100% flowering	Plant height (cm)	Stem length (cm)	Stem diameter (mm)
HEINZ	23.0	26.7	28.0	33.0	65.2	13.5	13.0
SHASTA	24.0	25.7	30.0	34.0	84.1	16.9	13.2
CRI-POO	23.0	23.0	28.0	32.7	68.6	11.6	13.6
OP-B149	19.3	28.0.	30.3	38.0	64.6	12.2	14.4
OP-B155	21.3	29.3	33.0	38.0	57.6	10.5	13.9
MEAN	22.1	26.5	29.9	35.1	68.0	12.9	13.6
LSD (0.05)	1.1	2.3	1.7	2.1	8.1	3.4	1.9
CV (%)	2.6	4.7	2.9	3.2	6.3	12.9	7.4

4.3 Morphological characteristics of five tomato varieties at KNUST.

Table 4.3 showed the phenotypic characteristics of the tomato varieties cultivated at KNUST. There were no significant differences among the varieties on the number of inflorescences per plants and number of flowers per inflorescences. Significant differences were found on the number of leaf under 1st inflorescence between (Shasta and OP-B149) only. The growth

habit for Heinz, Shasta and CRI-P00 was determinate. Except OP-B149 and OP-B155 which have large plant size, the rest of the varieties had intermediate plant size. OP-B149 and OP-B155 had round fruit shape whereas, Heinz, Shasta and CRI-P00 had heart fruit shape, slightly flattened and high round respectively (Table 4.4).

Table 4.3: Morphological characteristics of five tomato varieties studied at KNUST, Kumasi

Treatment	No. of inflorescence Per plant	No. of flowers per inflorescences	No. of leaf under the1st inflorescence
HEINZ	7.9	27.9	5.9
SHASTA	9.2	37.5	6.6
CRI-POO	8.8	19.8	6.2
OP-B149	8.4	31.4	5.5
OP-B155	7.4	28.8	6.4
MEAN	8.4	29.1	6.1
LSD(0.05)	4.4 (NS)	19.9 (NS)	0.9
CV (%)	27.8	36.4	8.5

Growth Habit: 2-Determinate and 3 Semi-determinate.
Plant Size: 5 – Intermediate and 7 – Large.
Leaf under 1st inflorescence: 3- Few and 7 – Many.
Fruit Shape: 5 – Heart shape, 4 – High rounded, 3 – Rounded, 2 – Slightly flattened.

Table 4.4 Growth Harvest, plant size and fruits shape of five tomato varieties studied at KNUST, Kumasi

Traits	**Description**	**Varieties**
Growth habit	Determinants semi determinant	HEINZ, SHASTA, CRI-POO OP-BI49, OP-BI55
Plant Size	Intermediate Large	HEINZ, SHASTA, CRI-POO OP-BI49, OP-BI55
Fruit shape	Heart High rounded Rounded Slight flattened	HEINZ CRI-POO OP-BI49, OP-BI55 SHASTA

4.4 Morphological characteristics of five tomato varieties at Kwadaso.

Table 4.5 shows the morphological characteristics of the tomato varieties cultivated at Kwadaso. Significant difference was found among all the varieties on the number of flowers per inflorescences. CRI-POO and OP-B155 also showed significant difference on the number of inflorescence per plant (Table 4.5). No significant differences were found on the number of leaf under 1st inflorescence. The growth habit for Heinz, Shasta and CRI-P00 was determinate (Table 4.6). The varieties had intermediate plant size (Table 4.5). OP-B149 and OP-B155 had round fruit shape whereas, Heinz, Shasta and CRI-P00 had heart fruit shape, slightly flattened and high round respectively (Table 4.6).

Table 4.5: Morphological characteristics of five tomato varieties studied at CRI-Kwadaso, Kumasi

Treatment	No. of inflorescences Per plant	No. of flowers per inflorescences	No. of leaf under the 1st inflorescence
HEINZ	5.9	25.0	5.5
SHASTA	6.1	29.6	6.3
CRI-POO	6.6	15.9	6.4
OP-B149	6.2	23.1	5.8
OP-B155	5.2	19.0	6.2
MEAN	6.0	22.5	6.0
LSD (0.05)	1.1	6.4	1.2
CV (%)	9.4	15.1	10.9

Growth Habit: 2-Determinate and 3 Semi-determinate.
Plant Size: 5 – Intermediate and 7 – Large.
Leaf under 1st inflorescence: 3- Few and 7 – Many.
Fruit Shape: 5 – Heart shape, 4 – High rounded, 3 – Rounded, 2 – Slightly flattened.

Table 4.6 Growth Harvest, plant size and fruits shape of five tomato varieties studied at CRI-Kwodaso, Kumasi

Traits	Description	Varieties
Growth habit	Determinants semi determinant	HEINZ, SHASTA, CRI-POO OP-BI49, OP-BI55
Plant Size	Intermediate Large	HEINZ, SHASTA, CRI-POO OP-BI49, OP-BI55
Fruit shape	Heart High rounded Rounded Slight flattened	HEINZ CRI-POO OP-BI49, OP-BI55 SHASTA

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4.5 Disease infection of five tomato varieties at KNUST.

Table 4.7 shows disease infection of five tomato varieties at KNUST. There were no significant differences among the treatments for late blight, bacterial wilt, and fusarium wilt and tomato fruit borer. There was significance difference on TYLCV incidence among the tomato varieties. OP-B149 recorded the highest incidence of TYLCV at KNUST. Heinz and Shasta however, gave the lowest TYLCV incidence. CRI-P00 was highly infested with Tomato Fruit borer (TFB). High incidence of blossom end rot was also recorded on the tomato varieties. Shasta and OP-B149 gave the highest and lowest infection of blossom end rot, respectively (Table 4.7).

Table 4.7: Disease incidence of five tomato varieties at KNUST.

Treatment	Early blight	Late blight	TYLCV incidence	Bacterial wilt
HEINZ	15.7	2.5	2.7	0.5
SHASTA	11.7	0.8	3.0	0.5
CRI-POO	18.0	1.2	4.3	0.5
OP-B149	13.3	0.5	7.3	0.5
OP-B155	12.3	0.5	5.7	0.5
MEAN	14.2	1.1	4.6	0.5
LSD (0.05)	8.5 (NS)	3.2 (NS)	4.6	
CV (%)	31.9	152.6	53.4	

BER: Blossom end rot.
TYLCV: Tomato yellow leaf curl virus.
TFB: Tomato fruit borer.
The disease scored were done using the tomato descriptor (AVDRC).

4.6 Disease infection of five tomato varieties CRI, Kwadaso.

Table 4.8 shows disease infection of five tomato varieties at CRI, Kwadaso. There were no significant differences among the treatments for late blight, bacterial wilt, and fusarium wilt and tomato fruit borer. Disease incidence of early blight was significant between Heinz and OP-B155, Heinz and CRI-P00. There was significance difference on TYLCV incidence among the tomato varieties (Table 4.8). OP-B149 recorded the highest incidence of TYLCV. CRI-P00 and Shasta gave the lowest TYLCV incidence. CRI-P00 was highly infested with Tomato Fruit borer (TFB). High incidence of blossom end rot was also recorded on the varieties.

Heinz and CRI-P00 gave the highest and lowest infection of blossom end rot (Table 4.8)

Table 4.8: Disease incidence of five tomato varieties at CRI, Kwadaso.

Treatment	Early blight	Late blight	TYLCV incidence	Bacterial wilt	Fusarium wilt	TFB	BER
HEINZ	32.0	0.5	8.5	1.2	1.8	1.2	110.7
SHASTA	15.0	1.2	5.8	1.8	1.8	0.2	57.3
CRI-POO	14.0	0.8	1.5	2.5	2.5	2.5	24.0
OP-B149	16.7	0.8	8.9	2.5	1.8	0.5	36.3
OP-B155	10.0	0.5	8.5	1.8	1.8	0.5	35.7
MEAN	17.5	0.8	6.7	1.9	1.9	1.2	52.8
LSD(0.05)	6.9	1.3	4.6	1.8	2.1	2.7	31.6
CV (%)	21.2	90.7	36.4	49.1	56.1	121.2	31.8

BER: Blossom end rot.
TYLCV: Tomato yellow leaf curl virus.
TFB: Tomato fruit borer.
The disease scored were done using the tomato descriptor (AVDRC).

4.7 Yield components of tomato varieties at KNUST

Table 4.9 shows the yield component of five tomato varieties at the different locations. There were significant differences in the varieties on the number of plants harvested, number of marketable and non-marketable fruits, average fruit weight (g), total marketable fruit weight (kg), brix, shelf life and yield per hectare. The highest yields were obtained on Shasta and CRI-P00 respectively. Heinz, produced the lowest yield (ton/ha). CRI-P00 and Heinz gave the highest and lowest average fruit weight, respectively. Heinz and Shasta recorded the highest number of days (40 days) to store whereas OP-B149 and OPB-155 took less number of days (22 days) to store. The highest brix was found in Heinz and Shasta whereas CRI-P00, OPB-155 and OPB-149 gave the lowest brix content.

Table 4.9: The yield components of five tomato varieties at KNUST.

Treatment	No. of Plant harvest	No. of MKT fruits	No. of NMKT fruits	Average fruits wt. (g)	Total MKT wt. (kg)	t/ha	Brix	Shelf Life/days
HEINZ	79.0	189	148.7	298	13.8	11.5	3.4	40.0
SHASTA	103.3	465	149.7	344	35.6	29.7	3.4	40.0
CRI-P00	88.3	396	62.0	579	35.4	29.4	2.9	28.0
OP-B149	65.3	158	45.3	329	16.2	13.5	3.2	22.0
OP-B155	59.7	138	71.0	334	13.9	11.6	2.9	22.0
MEAN	79.1	269.3	95.3	376.8	22.9	19.2	3.2	30.4
LSD (0.05)	36.1	147.9	51.4	228.2	13.8	15.3	0.4	
CV (%)	17.5	29.2	29.6	32.2	31.9	43.9	7.1	

MKT: Marketable
NMKT: Non-marketable

4.8 Yield components of tomato varieties at CRI, Kwadaso

Table 4.10 shows the yield component of five tomato varieties at Kwadaso. There were significant differences in the varieties on the number of plants harvested, number of marketable and non-marketable fruits, average fruit weight (g), total marketable fruit weight (kg), brix, shelf life and yield per hectare. The highest yields were obtained on CRI-P00 and Shasta respectively. Heinz however, produced the lowest yield (ton/ha). CRI-P00 and Heinz gave the highest and lowest average fruit weight respectively. Heinz and Shasta recorded the highest number of days (40 days) to store whereas OP-B149 and OPB-155 took less number of days (22 days) to store. The highest brix was obtained by Heinz and Shasta whereas CRI-P00, OPB-155 and OPB-149 gave the lowest brix content.

Table 4.10: The yield components of five tomato varieties at CRI, Kwadaso.

Treatment	No. of Plant harvest	No. of MKT fruits	No. of NMKT fruits	Average Fruit Wt. (g)	Total MKT Wt. (kg)	t/ha	Brix	Shelf life
HEINZ	76.7	144.0	112.3	157.6	5.7	4.8	3.6	40.0
SHASTA	93.3	239.0	59.0	257.6	13.1	10.9	3.6	40.0
CRI-P00	88.0	277.0	27.7	465.4	21.4	17.8	3.0	28.0
OP-B149	51.0	105.7	38.0	266.6	6.9	5.8	2.9	22.0
OP-B155	46.7	92.7	37.7	246.2	6.6	5.5	3.6	22.0
MEAN	71.1	171.7	54.9	278.7	10.8	8.9	3.4	30.4
LSD (0.05)	10.6	49.2	32.5	72.0	5.2	5.5	0.6	
CV (%)	7.9	15.2	31.5	13.7	25.8	33.9	9.9	

MKT: Marketable
NMKT: Non-marketable

4.9 Sensory evaluation on five tomato varieties.

Figure ~~6~~4.1 shows various preparations of soup, stew and salad using different tomato varieties at Kwadaso. There were significant differences in the Soup and Stew preparations on their appearance and flavour respectively. Variation on the Soup appearance was found between OP-B149 and Shasta whereas Heinz and OP-B155 also gave differences in the Stew flavour. Nevertheless, no varied differences were established among the other sensory characters assessed.

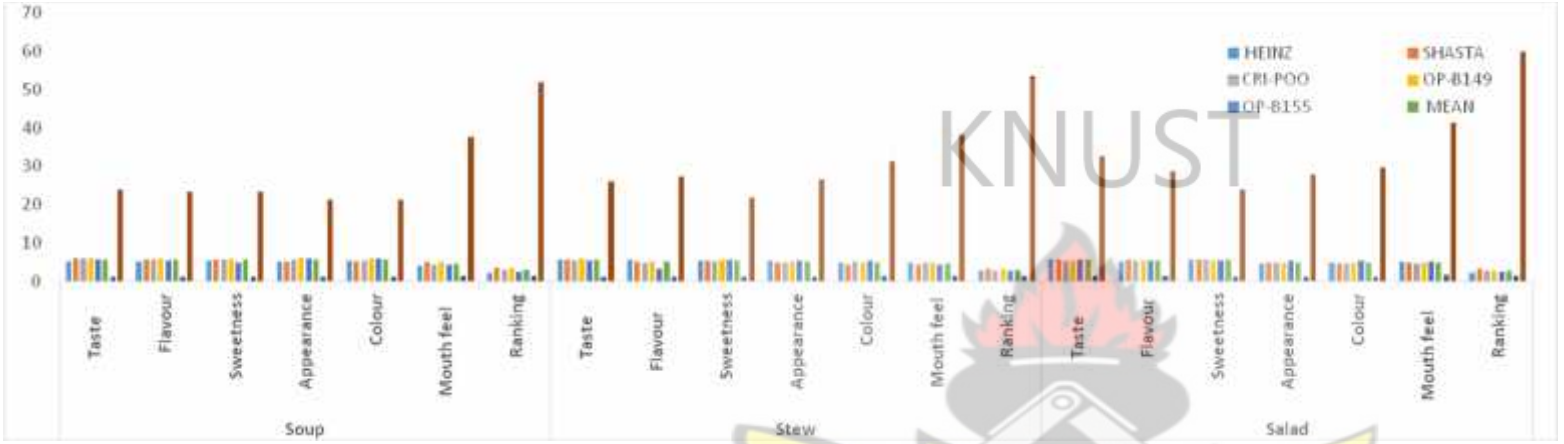

Figure 4.16: Preparations of Soup, Stew and Salad using different tomato varieties

4.10 Evaluation of SSR markers in tomato varieties using dendrogram.

The agglomerative hierarchical clustering dendrogram illustrates the relationship among the accessions (Fig. ~~7~~4.2). At a similarity coefficient of 0.85, two main groups were obtained. Cluster A had only one variety (OP-B149) while cluster B had four varieties (OP-B155, CRI-P00, Shasta and Heinz). At a coefficient of 0.88, cluster B had three sub-clusters BI (Heinz), BII (Shasta) and BIII (OP-B155 and CRI-P00). At a coefficient of 0.90 varieties OP-B155 and CRI-P00 were identified as the most genetically related varieties (very identical).

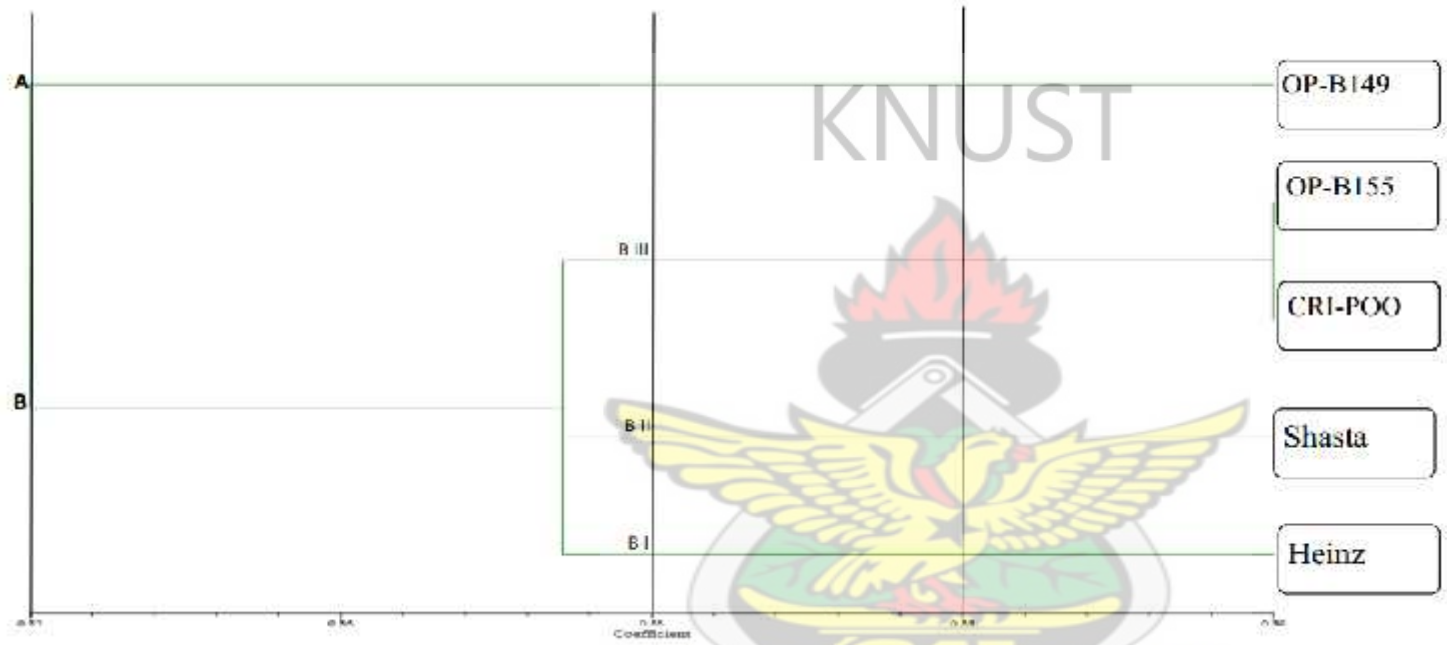

Figure 4.27: Dendrogram produced from NTSYS2.2 based on SSR molecular marker of five (5) tomato varieties.

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4.11 Assessment of SSR markers for evaluating genetic diversity in tomato varieties.

Table 10 indicate Allele frequency, Number of Allele, Gene diversity, Heterozygosity and Polymorphic Information Content (PIC) values generated from SSR data. Out of the 15 primers, 14 primers gave polymorphic bands and therefore were considered for further analysis. The number of alleles ranged from 2.00 (SSR2), (SSR3) to 6.00 (SSR9), SSR11) alleles per locus with a mean value of 4.07 alleles per locus. The Polymorphic Information Content (PIC) values also ranged from 0.31 (SSR2) to 0.77 (SSR9) with an average of 0.59. The most polymorphic primers was SSR9, SSR11, and SSR4 based on PIC values. The allele frequency of the primers indicate that, SSR5 was not polymorphic and was therefore dropped. The rest were all polymorphic in character. Gene diversity was high ranging from 0.38 (SSR2) to 0.80 (SSR9) with a mean of 0.61. However, SSR8, SSR10 and SSR13 had the same Gene diversity of 0.66 with a mean value of 0.61 for all.

Table 10: Allele frequency, Number of Allele, Gene Diversity, Heterozygosity and Polymorphic Information Content (PIC) values generated from SSR data.

Marker	Allele frequency	Allele No.	Gene diversity	Heterozygosity	PIC
SSR1	0.7500	3.0000	0.4063	0.5000	0.3706
SSR2	0.7500	2.0000	0.3750	0.5000	0.3047
SSR3	0.5000	2.0000	0.5000	1.0000	0.3750
SSR4	0.2500	4.0000	0.7500	1.0000	0.7031
SSR6	0.5000	4.0000	0.6563	0.5000	0.6050
SSR7	0.6000	3.0000	0.5400	0.8000	0.4662
SSR8	0.4000	4.0000	0.6600	1.0000	0.5958
SSR9	0.3000	6.0000	0.8000	1.0000	0.7716
SSR10	0.4000	4.0000	0.6600	1.0000	0.5958
SSR11	0.3000	6.0000	0.7800	0.8000	0.7482
SSR12	0.6000	4.0000	0.5800	0.6000	0.5350
SSR13	0.5000	4.0000	0.6600	0.4000	0.6102
SSR14	0.5000	3.0000	0.6200	0.6000	0.5478
SSR15	0.6000	5.0000	0.6000	0.6000	0.5700
Mean	0.4607	4.0714	0.6134	0.7357	0.5942

Allele frequency, Number of Allele, Gene Diversity, Heterozygosity and Polymorphic Information Content (PIC) values were calculated using Power Marker V3.25

Figure 4.3~~8~~: Primer 1 and primer 2 used to amplify the five tomato varieties.

M= Marker, **T149 =** OP-B149, **T155=** OP-B155, **TC** = CRI-POO, **TS** = Shasta, **TH** = Heinz, **bp** = base pair

CHAPTER FIVE

5.0 DISCUSSION

5.1 Agronomic Characteristics of Tomato Varieties.

Agronomic characters of the tomato varieties showed significant differences in almost all the genotypes across locations. This may be due to differences in genetic and environmental conditions. This is expected since different genotypes perform differently in same environment (Blay *et al.* 1999). Variations in the climatic conditions during the experiments provided sufficient evidence for the variation that existed in the performance of the varieties. Understanding the performance of varieties is useful for breeding purpose, assortment efficiency and prediction of their performances. Poor plant establishment in some of the tomato varieties can be attributed to their inability to withstand diseases and pests during the growing seasons at the experimental areas. Generally, flowering appeared early in all the accessions, since flowering in tomato usually starts 50 to 65 days after sowing (Sinnadurai 1992). Tomato varieties varied in plant heights. This confirmed Messian (1992) who indicated tomato plant height may vary up to 2 m tall. The variation in temperature during the experiment may have also caused stem length and stem diameter to reduce across locations as reported by Goldhamer and Fereres (2001). Plants that experience water stress generally show larger daily stem contractions and lower rates of stem growth compared to well-watered plants (Goldhamer and Fereres, 2001).

5.2 Morphological characteristics of tomato varieties

Varied differences on the number of inflorescence per plant and flowers per inflorescence may be due to environmental conditions at Kwadaso. The poor number of inflorescences per plant observed in this study could, therefore, be attributed to the high temperatures which probably caused a decrease in pollen fertility as observed by George (1985). In addition, the

high incidence of flower abscission observed further accounted for the poor yield. According to Norman (1992), tomato performs best at day temperature of 23.9 to 29.4°C and night temperature between 15.6 and 21.1°C. Extreme temperatures during flowering may cause tomato pollen abscission, bud drop, failure of anther to dehisce, and other flower abnormalities resulting in low fruits set (El – Ahmadi and Stevens, 1979). Similarly, it was found that the number of leaves formed before initiation of the first inflorescence may had decreased with increased light intensity as observed by Kinet (1977). The tomato varieties showed two growth patterns, determinate or semi determinate. This growth habit might have been selected for over the years because it rarely requires staking and pruning. Likewise, it is able to combine large numbers of fruit with many plants per unit space, which is an indicator for high yield (Osei *et al*., 2010). Breeders could incorporate determinate growth habit in their tomato improvement program. Tomato varieties that had semi-determinate growth habit could give longer harvesting period. This is an advantage if prices fluctuate. The variation in plant size at the locations may be due to climatic conditions as also observed by George (1985). The difference in fruit shape may had been due to time of establishment of flowers and size of ovary. According to Van der Knaap *et al.,* (2002), the major loci that have been identified as contributing to an elongated shape in tomato are sun and ovate. Dissimilarity in fruit shape is the result of differential growth processes which probably occur during formation of the ovary, or after anthesis during the formation of the fruit.

5.3 Disease infection of tomato varieties.

The reason attributed to low disease infections may be due to the good agronomic practices and low pest pressure during the growing season for the experiment at both locations. These agronomic practices were in agreement with Joey, (2009) which say that removal of plants/fruits with initial symptoms may slow the spread of the diseases. The high incidence of early blight and TYLCV among the varieties may also be attributed to high temperature and

humidity that occur during the experimental period. High frequency of blossom end rot was physiological disorder that occurred during the experiment. According to Joey, (2009) this disorder is a calcium deficiency in the developing of the fruit. Moreover, extreme fluctuations in moisture, root pruning from nearby cultivation and excessive ammonia (NH_4^+), nitrogen, potassium, or magnesium fertilization can also increase the chances of blossom end rot, especially early in the season (Joey, 2009).

5.4 Yield components of tomato varieties.

The significant response of tomato varieties to yield and quality characters may be due to the genetic makeup, status of water and oxygen during the growing period of these varieties. The oxygen deficiency restricts root respiration and negatively affects water and nutrient uptake. This eventually reduces the yield and its quality. Raviv *et al.* (2004) reported that responses to low yield and quality may also be attributed to the poor rainfall, high day and night temperatures during the experimental period. Studies have shown that tomato yields are drastically reduced in the dry season by 6 to 45% due to low moisture supply, depending on the varieties and growing conditions (Blay *et al.*, 1999; Norman, 1974; Sinnadurai and Doku 1976; Villareal, 1981. The high incidence of blossom end rot outbreak may also be a contributing factor. According to Joey, (2009) extreme fluctuations in moisture and insufficient soil calcium can increase blossom end rot and as a result affect the yields. The shelf life of tomato usually last for 45 days after harvesting (http://www.deccanherald.com /content/50265/gm-tomato-gives-45-day.html). However, shelf life on the five varieties were below the 45 days. This may have been the lack of firmness of these varieties (Gerasopoulos *et al.*, 1996).

Nevertheless, supplemental Ca^{2+} applied immediately before or just after harvest has been revealed to increase fruit firmness and extend storage life (Lopez-Lefebre *et al.*, 2000).

5.5 Sensory evaluation on five tomato varieties.

Variations in appearance and flavour of the five tomato varieties may have been associated with volatile and non-volatile compounds (Krumbein *et al.*, 2004). However, appearance and flavour of tomato varieties not only results from the sum of volatile or non-volatile compounds, but also depends mostly on their relations as well (Petro-Turza, 1987). Therefore, to improve the appearance and flavour of tomatoes, harvesting of tomatoes should not be done at the mature-green stage (Kadar *et al.*, 1977).

5.6 Evaluation of SSR markers in tomato varieties using dendrogram.

The aim of this study was to characterize tomato varieties using SSR markers. Even though, some SSR markers have been developed, their availability is limited. Recently, SGN has developed 609 SSR markers and assayed on *Solanum lycopersicum* and *Lycopersicon pennellii* (Frary *et al.*, 2005). Consequently, 15 SSR markers used for genetic characterization among the five tomato varieties revealed some distinction and similarity level among them. The similarity level were due to the narrow genetic diversity, as has been previously stated by Suliman Pollatschek *et al.*, (2002) and He *et al.*, (2003).

The genetic diversity of the accessions studied was evaluated by using 14 polymorphic SSR primers. In essence, the allele frequency analysis calculates two common measure of variation for each locus namely, expected heterozygosity and polymorphic information content (PIC). The expected heterozygosity measure is helpful in establishing the informativeness of a locus. Loci with expected heterozygosity of 0.5 or less are not very useful for large-scale parentage analysis (Otoo *et al.*, 2009). The results of observed heterozygosity of the loci was greater than 0.5, in all the markers used for the study, expect in

SSR13 (0.40) signifying that a good parentage analysis can be obtained from the molecular analysis. This observation also illustrates that, at a single locus, any two alleles, chosen at random from the population are different from each other (IPGRI and Cornell University, 2003). Additionally, the high observed heterozygosity values in this study confirm the heterozygote nature of most of the accessions studied (Obidiegwu *et al.,* 2009). Nevertheless, the average heterozygosity over all loci therefore estimates the extent of genetic variability in the population. The mean heterozygosity value of 0.74 reveals that there was some degree of genetic variation among the population (IPGRI and Cornell University, 2003).

Moreover, the efficiency of each primer was estimated by the number of alleles and discriminating power was calculated by the assessment of the polymorphic information content. PIC is regarded as one of the important features of molecular markers and can be used to evaluate the differentiation ability of the markers within the population (Junjian *et al.*, 2002). PIC is a measure of informativeness related to expected heterozygosity and is calculated from allele frequencies (Norman *et al.*, 2012). Such measurement is useful in linkage mapping studies. The results from the allelic frequency analysis generally implied that the loci revealed high polymorphism verified by elevated PIC values (0.31-0.77) (Table 6). Thus, the SSR markers used were efficient in discriminating the species. The amount of PIC is a function of detected alleles and the distribution of their frequency (Moghaddam, M. and Alikhani, M. 2009). Thus markers with more alleles and low allele frequency had larger PIC as found in SSR9 (6 alleles and the highest PIC of 0.77 followed by SSR11 (6 alleles and the PIC of 0.74) respectively indicating a better distinction of the accessions. These results confirmed the utility of the PIC as a measure of the capacity of a marker to discriminate among closely related individuals as pointed out by Prevost and Wilkinson, (1999) and Escandon *et al.* (2007). PIC values demonstrated that the SSRs used in the study were highly informative. The mean PIC value recorded in this study however differs from results obtained

from previous studies by Bredemeijer *et al.* (2002) who reported that the number of alleles per locus ranged from 2 to 8 with an average of 4.7 alleles per locus in 521 tomato varieties. He *et al.* (2003) found 2 to 6 alleles for each locus after testing 65 SSR loci on 19 tomato accessions. Similarly, Garcia-Martinez *et al.* (2006) reported that number of SSR alleles detected in 48 tomato accessions ranged from 2 to 10 alleles for the 19 SSR markers. In addition, PIC mean values (0.59) for this study was greatest than those recorded He *et al.,* (2003) (0.37), Bredemeijer *et al.,* (2002) (0.40), Frary *et al.,* (2005) (0.39) and less Garcia-Martinez *et al.,* (2006) (0.78). The result of this study therefore showed that all the primers were highly informative and can be used for genetic diversity studies and the study of phylogenetic relationship.

The markers SSR1, SSR2, SSR7, SSR12 and SSR15 (Table 6) had the highest frequencies of 0.75, for SSR1 and SSR2, 0.60, for SSR7, SSR12 and SSR15 respectively, while SSR4, SSR9, SSR11 and SSR8, SSR10 (Table 6) had the lowest frequencies of the predominant allele (0.25, for SSR4, 0.30, for SSR9 and SSR11 and 0.40 for SSR8 and SSR10 respectively). Low frequency of the predominant allele reveals the suitable allelic distribution among the accessions (Priolli *et al.,* 2002). SSR markers with the higher number of alleles per locus showed the lowest frequency of the predominant allele, thus, markers with lower frequency of the predominant allele have more differentiation ability than other markers.

Large number of alleles per locus observed (4.07, on average, varying from 2 to 6 alleles) in this study is an indication of considerable allelic variants per locus (genetic diversity present) among the tomato accessions under investigation (Moghaddam, M. and Alikhani, M. 2009).

A gene is said to be polymorphic if the frequency of one of its alleles is less than or equal to 0.95 or 0.99 (IPGRI and Cornell University, 2003). Results observed from allelic frequency analysis proved that all the 14 out of the 15 primers were polymorphic. No rare alleles

(alleles with allelic frequencies of less than 0.005) were obtained. This was probably due to the genetic closeness of the genotypes studied. Gene diversity values of 0.61 on average were also observed. This demonstrates genetic polymorphism in the tomato germplasm studied.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSION

- The genetic and climatic conditions were the major factors that brought about variation in the tomato varieties based on their agronomic and morphological characters and yields across the study locations.
- Shasta emerged exceptional or outstanding among the tomato varieties based on the yield, other agronomic characteristics and post-harvest including shelf life.
- OP-B149 and Heinz had the highest stem diameter which make them have good advantage to tolerate water stress.
- The varieties studied were either determinate or semi determinate.
- The varieties exhibited different fruit shapes such as rounded, heart shaped, slightly flattened and high rounded.
- Disease and pest incidence were low and could be due to the growing season for the studies
- Shasta and Heinz took forty (40) days to store given the highest shelf life for the varieties studied.
- The sensory evaluation test also revealed variation in soup appearance for OP-B149, Shasta and stew flavour for Heinz and OP-B155.
- Agglomerative hierarchical clustering and SSRs markers were effective in assessing varieties and molecular diversity within the tomato germplasm collection.
- Molecular markers grouped the accessions into four (4) main clusters (A, B, C and D) with B having two accessions and A, C and D one accession each (Fig. 7).

- Primers SSR1, SSR2, SSR7, SSR12 and SSR15 recorded the highest number of alleles detected per locus.
- Among the primers used for the DNA fingerprinting, SSR9 recorded the highest PIC.
- The introduced tomato accessions including the check used in the study were genetically variable and therefore clustered in groups based on their close relationships or associations.
- The objectives was to provide farmers with better and superior varieties of tomatoes: Shasta and CRI-POO could be selected in view of their superior yields of nearly 30 t/ha.

6.2 RECOMMENDATION

- With the diversity observed in the varieties studied based on the phenotypic and molecular analysis, breeders can incorporate that in their programs.
- Varieties with most distinct characters may be good for crop improvement programs.
- Breeders could incorporate determinate growth habit in their tomato improvement program.
- Shasta is recommended for tomato breeders following the fact that, it is high yielding has good shelf life and soup appearance.

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APPENDIX 1: Sensory Evaluation form for Tomato Soup

Product: Tomato soup

Name:…….. Date:…… Codes:…001……

Gender: M / F Age:……

Instruction:

Please, taste each of the five (5) samples of Tomato soup before you in the order indicated. Give your perception on the Taste, Flavour, Sweetness, Appearance, Colour and Mouth-feel of each of the product on the scale below using 7 to 1.

Scale	**Score**
Like extremely	7
Like moderately	6
Like slightly	5
Neither like nor dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike extremely	1

Tomato soup	**Taste**	**Flavour**	**Sweetness**	**Appearance**	**Colour**	**Mouth-feel**
100						
200						
300						
400						
500						

APPENDIX 2: Sensory Evaluation Form for Tomato Soup

Product: Tomato soup

Name:…….. Date:……... Codes:…002……

Gender: M / F Age:……

Instruction:

In front of you are five (5) coded samples of Tomato stew. Observe the samples in the order presented on the Score-card. Describe your perception on the chalkiness on the scale below using 5 to 1.

Scale	**Ranking**
Weak	5
Moderately Light	4
Light	3
Moderately strong	2
Strong	1

	Codes				
Products:	100	200	300	400	500
Rank Assigned	……	……	……	.....	.....

APPENDIX 3: Sensory Evaluation form for Tomato Stew

Product: Tomato stew

Name:…….. Date:…… Codes:…003……

Gender: M / F Age:……

Instruction:

Please, taste each of the five (5) samples of Tomato stew before you in the order indicated. Give your perception on the Taste, Flavour, Sweetness, Appearance, Colour and Mouth-feel of each of the product on the scale below using 7 to 1.

Scale	**Score**
Like extremely	7
Like moderately	6
Like slightly	5
Neither like nor dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike extremely	1

Tomato soup	**Taste**	**Flavour**	**Sweetness**	**Appearance**	**Colour**	**Mouth-feel**
100						
200						
300						
400						
500						

APPENDIX 4: Sensory Evaluation form for Tomato Stew

Product: Tomato stew

Name:…….. Date:……... Codes:…004……

Gender: M / F Age:……

Instruction:

In front of you are five (5) coded samples of Tomato stew. Observe the samples in the order presented on the Score-card. Describe your perception on the chalkiness on the scale below using 5 to 1.

Scale	**Ranking**
Weak	5
Moderately Light	4
Light	3
Moderately strong	2
Strong	1

	Codes				
Products:	100	200	300	400	500
Rank Assigned	……	……..	…….	…….	……..

APPENDIX 5: Sensory Evaluation form for Tomato Salad

Product: Tomato salad

Name:…….. Date:…… Codes:…005……

Gender: M / F Age:……

Instruction:

Please, taste each of the five (5) samples of Tomato salad before you in the order indicated. Give your perception on the Taste, Flavour, Sweetness, Appearance, Colour and Mouth-feel of each of the product on the scale below using 7 to 1.

Scale	**Score**
Like extremely	7
Like moderately	6
Like slightly	5
Neither like nor dislike	4
Dislike slightly	3
Dislike moderately	2
Dislike extremely	1

Tomato soup	**Taste**	**Flavour**	**Sweetness**	**Appearance**	**Colour**	**Mouth-feel**
100						
200						
300						
400						
500						

APPENDIX 6: Sensory evaluation form for tomato salad

Product: Tomato salad

Name:…….. Date:……... Codes:…006……

Gender: M / F Age:……

Instruction:

In front of you are five (5) coded samples of Tomato salad. Observe the samples in the order presented on the Score-card. Describe your perception on the chalkiness on the scale below using 5 to 1.

Scale	**Ranking**
Weak	5
Moderately Light	4
Light	3
Moderately strong	2
Strong	1

	Codes				
Products:	100	200	300	400	500
Rank Assigned	……..	……..	……..	……..	……..

APPENDIX 7: CTAB method for extraction of DNA from leaves

(Takrama, 2000 CRIG)

PROTOCOL

1. Grind 20mg of fresh sample in 2.0ml microtubes to fine powder (CTAB buffer can be added), with liquid Nitrogen.

2. Add **800µl** of 2% CTAB with 0.1 % of mercaptoethanol.

3. Incubate in a sand bath at 65oC for 30 min with intermittent vortexing.

4. Cool sample at room temperature and add equal volume (**800 µl**) of chloroform isoamyl alcohol (24:1).

Mix by several inversions of the tube.

5. Centrifuge at 14000 rpm for 15min.

6. Transfer the aqueous phase of the sample into a clean 1.5ml tube.

7. Precipitate nucleic acids by adding two thirds volume of ice cold isopropanol (**400 µl**) and shake gently. Keep on ice for 30 min. Precipitation can be enhanced by storing at -20°C for 8 hours or overnight.

8. Centrifuge at 14000 rpm for 5 min to pellet nucleic acids.

9. Decant the isopropanol and wash pellet with **500 µl** of washing buffer on a rocking surface for 15 min and centrifuge at 6000rpm for 4min.

10. Decant washing buffer and wash pellet in **400 µl** (80%) ethanol then centrifuge at 6000rpm for 4 min.

11. Decant ethanol and dry pellet in vacuum or at 37°C for 10 min or until the smell of ethanol is no longer detectable.

12. Suspend DNA in **50µl** 1X TE buffer and centrifuge at high speed for 30sec to remove all insolubles.

13. Prepare 1.0% or 0.8% Agarose gel with (**3µl)** 0.003% Ethidium bromide or (5µl**)** 0.005% gel red solution.

14. Pipette **5µl** sample and add **1µl** loading buffer (6 Bromophenol blue).

15. Load the sample in the wells on gel submerged in 1X TAE buffer.

16. Run sample at (90 to 120) volts for forty five minutes (45mins).

17. Photograph under UV light

APPENDIX 8: Sequence of polymorphic primers.

SSR Name	Name	Left primer sequence	sequence
	Right primer sequence		
TGS0001F/TGS0001R		GCGACCCTCTATTGAACTTGAAGAC	
		ACAAATCAAAGGAACAATTTCAA	
TGS0002F/TGS0002R		CAAACGTGTTCGAGTTCGTG	CAAACGTGTTCGAGTTCGTG
		CCACACAATAAAGACAGAAAAATG	
TGS0003F/TGS0003R		ATGCATGCGTGTGTGTTGTA	ATGCATGCGTGTGTGTTGTA
		GTGTGTGTGTGTGTGTGTGTGT	
TGS0004F/TGS0004R		GCAATTTATTTTCATTTGTTATACCGG	GCAATTTATTTTCATTTGTTATACCGG
		ACCGAGACTCCTGGCTCATA	
TGS0005F/TGS0005R		GACAAAAATTTTCCACACGGC	GACAAAAATTTTCCACACGGC
		TCTCTTATAATTTTGTTGAGTCTCTGA	
TGS0006F/TGS0006R		GTCGCATAAATATGGACAACGA	GTCGCATAAATATGGACAACGA
		TTTTTAAAATACCATTCCAGAAAAA	
TGS0007F/TGS0007R		GTGGATTCACTTACCGTTACAAGTT	CATTCGTGGCATGAGATCAA
TGS0008F/TGS0008R		GCGGTGTGAAATACAACAAGACG	GCGGTGTGAAATACAACAAGACG
		CTCGACAAGCTAATTTCTGGG	
TGS0009F/TGS0009R		GCGAAGCAAAAGAAAATTGGG	GCGAAGCAAAAGAAAATTGGG
		CACCACGAAGGCTGTTGTTA	
TGS0010F/TGS0010R		TTGAAAAGCTGAAAAGTCAATCA	TTGAAAAGCTGAAAAGTCAATCA
		GAGAGGTGCCACATCACCTT	
TGS0012F/TGS0012R		GTCCCTACCCCACAAATTGAA	GTCCCTACCCCACAAATTGAA
		AGGTACAACTCACCTCCCCC	
TGS0013F/TGS0013R		GGTGGACATATGAGAAGACCTTG	GGTGGACATATGAGAAGACCTTG
		TCATTTTCCAATGGTGTCAAA	
TGS0014F/TGS0014R		GTGAAGACGAAAAACAAGACGA	GTGAAGACGAAAAACAAGACGA
		CCTTCCCCTTTTGTCTCTCC	
TGS0020F/TGS0020R		TCTTTCAACTTCTCAACTTTGGC	TCTTTCAACTTCTCAACTTTGGC
		GCCGACTTCAAAAACTGCTC	
TGS0023F/TGS0023R		GTCCAAATTAAAAACTAACCGCA	GTCCAAATTAAAAACTAACCGCA
		TTTCCAAAATGACCTAGCGG	

APPENDIX 9: Polymerases Chain Reaction (PCR) master mix.

The PCR master mix were from the KAPA 3G, and the standard reaction setup is provided below:

	Final conc.	50 µl rxn	10 µl rxn	№. Of rxn	Master mix
PCR water up to 50 µl	-	as required	3.22 µl	80	257.6
KAPA Plant PCR Buffer	1X	25 µl	5.0 µl	80	400.0
F – Primer (10 µM)	0.3 µM	1.5 µl	0.3 µM	16	4.8
R – Primer (10 µM)	0.3 µM	1.5 µl	0.3 µM	16	4.8
DNA polymerase (2.5u/µl)	1 u/50 µl	0.4 µl	0.08 µl	80	6.4
Template DNA (0.5 mm or 0.35 mm)	1 – 10 ng	as required	1.0 µl	-	-
PCR Enhancer (100X) OPTIONAL	as required (0 – 1X)	as required	0.1 µl	80	8.0

APPENDIX 10: Primer 3

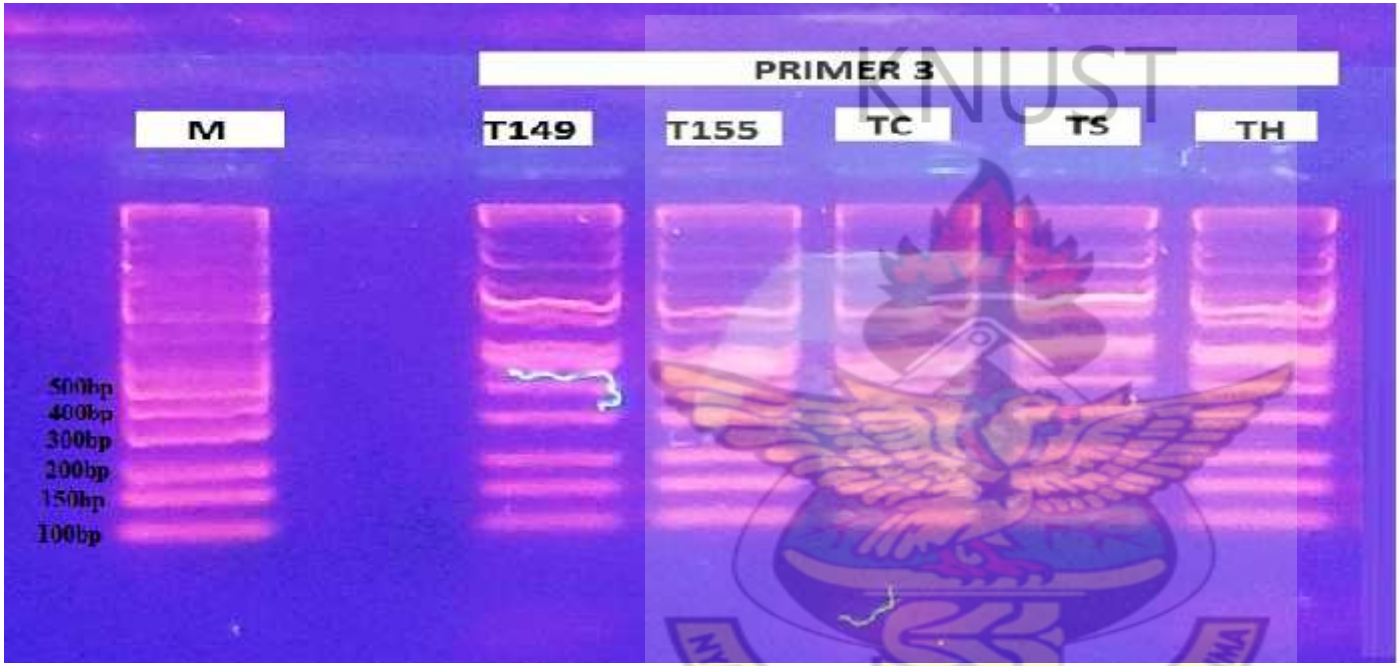


Figure 9 Primer 3

M= Marker, **T149 =** OP-B149, **T155=** OP-B155, **TC** = CRI-POO, **TS** = Shasta, **TH** = Heinz, **bp** = base pair

APPENDIX 11: Primers 4, 5 and 6

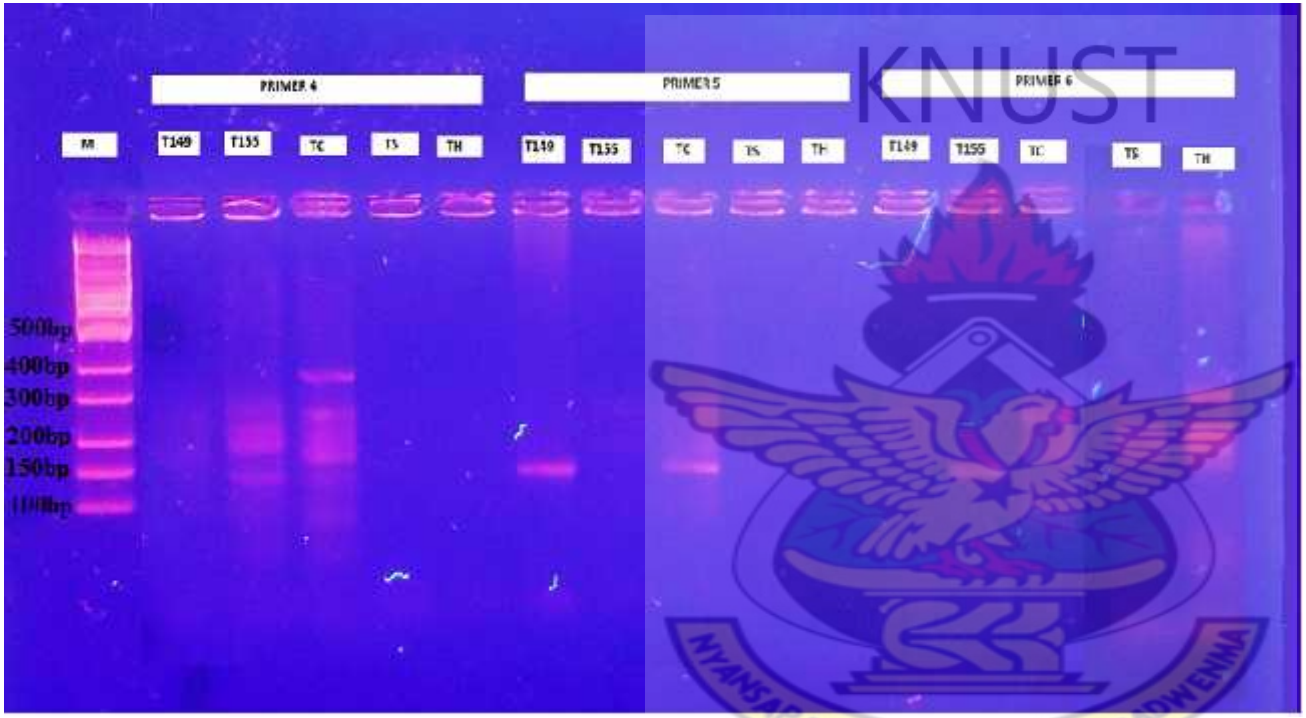


Figure 10 Primer 4, primer 5 and primer 6.

M= Marker, **T149 =** OP-B149, **T155=** OP-B155, **TC** = CRI-POO, **TS** = Shasta, **TH** = Heinz, **bp** = base pair

APPENDIX 12: Primers 7, 8, 9, 10 and 11

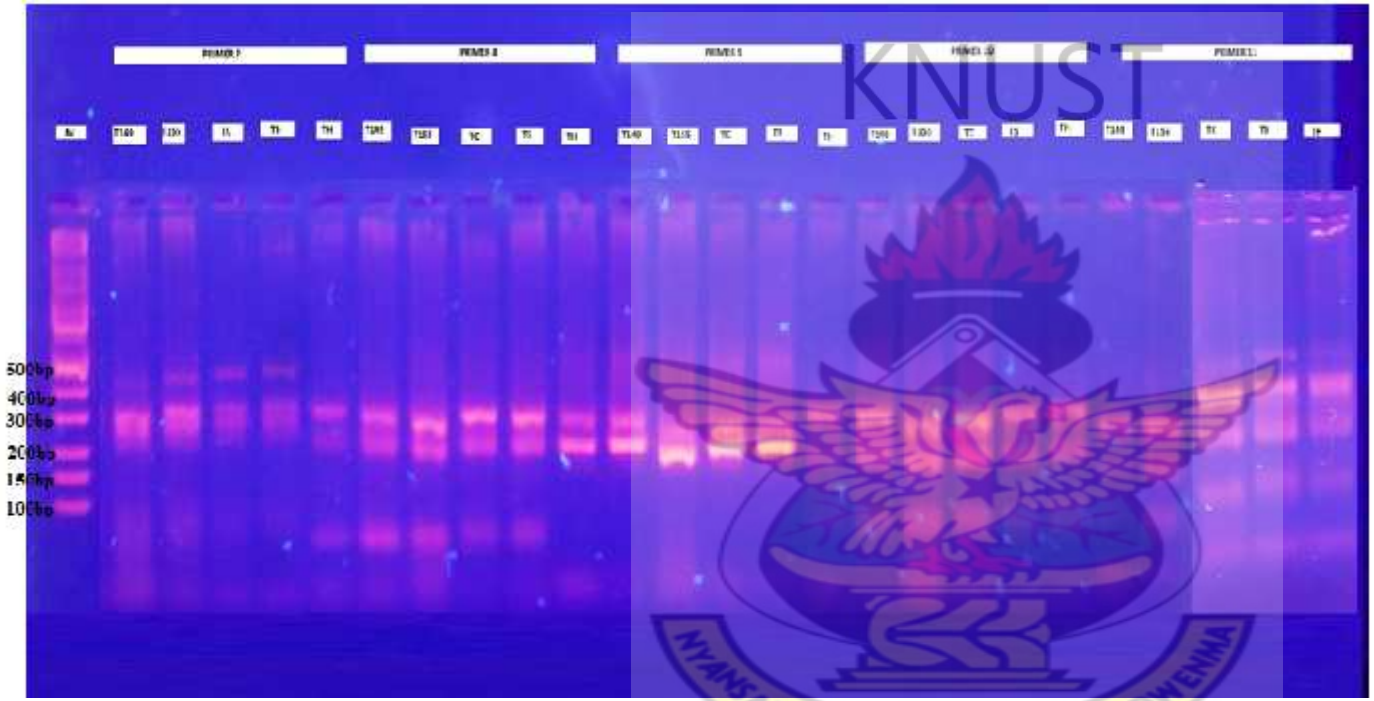


Figure 11 Primer 7 primer 8, primer 9, primer 10 and primer 11.

M= Marker, **T149 =** OP-B149, **T155=** OP-B155, **TC** = CRI-POO, **TS** = Shasta, **TH** = Heinz, **bp** = base pair

APPENDIX 13: Primers 12, 13 and 14

Figure 12 Primer 12 primer 13 and primer 14.

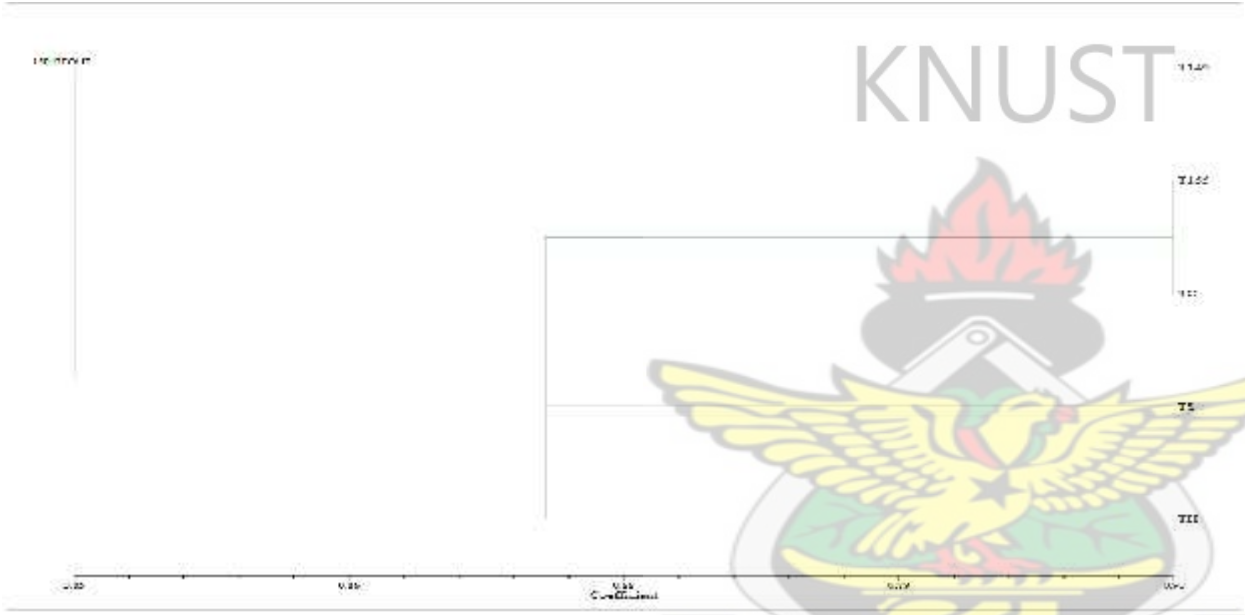
M= Marker, **T149 =** OP-B149, **T155=** OP-B155, **TC** = CRI-POO, **TS** = Shasta, **TH** = Heinz, **bp** = base pair

APPENDIX 14: Primer 15

Figure 13 Primer 15

M= Marker, **T149 =** OP-B149, **T155=** OP-B155, **TC** = CRI-POO, **TS** = Shasta, **TH** = Heinz, **bp** = base pair

APPENDIX 15: Dendrogram produced from NTSYS2.2 based on SSR molecular marker of five (5) tomato varieties.



APPENDIX 16: Mean, standard error, range, least significant difference and coefficient of variation were analysed for twelve quantitative traits.

	KNUST				CRI-KWADASO			
Traits	**Mean ± S.E**	**Range**	**LSD (5%)**	**CV (%)**	**Mean ± S.E**	**Range**	**LSD (5%)**	**CV (%)**
Plant per plot	22.27 ± 0.75	21.00-23.67	1.74	4.1	22.13±0.47	24.00-19.00	1.05	2.6
Days to first flower	26.53 ± 1.18	24.33-29.00	2.73	5.5	26.53±1.01	29.33-23.00	2.33	4.7
50% flowers	29.47 ± 0.59	29.33-30.67	1.35	2.4	29.87±0.72	33.00-28.00	1.65	2.9
100% flowers	34.53 ± 1.07	31.67-38.00	2.47	3.8	35.13±0.92	38.00-32.67	2.12	3.2
Stem length (cm)	12.44 ± 0.64	9.67-14.99	1.49	6.3	12.94±1.37	16.93-10.53	3.15	12.9
Stem diameter (mm)	14.54 ± 1.55	13.48-16.16	3.58	13.1	13.61 ± 0.83	14.37-13.03	1.91	7.4
Plant height (cm)	87.80 ± 6.46	81.10-98.20	14.89	9.0	68.00±3.50	84.10-57.60	8.07	6.3
Inflore. per plant	8.36 ± 1.89	7.43-9.20	4.38	27.8	6.01 ± 0.46	6.60 - 5.20	1.07	9.4
Flower per inflore.	29.10 ± 8.64	19.80-37.50	19.92	36.4	22.51± 2.78	29.57-15.90	6.42	15.1
Leaf under 1st inflore.	6.13 ± 0.43	5.53-6.63	0.98	8.5	6.01± 0.53	6.43-5.50	1.23	10.9
Growth habit	2.40 ± 0.00	2.00-3.00	*	0.0	2.00 ± 0.00	2.00-2.00	*	0.0
Plant size	5.80 ± 0.00	5.00-7.00	*	0.0	5.00 ± 0.00	5.00-5.00	*	0.0

APPENDIX 17: Mean, standard error, range, least significant difference and coefficient of variation were analysed for seven quantitative traits.

	KNUST				CRI-KWADASO			
Traits	**Mean ± S.E**	**Range**	**LSD (5%)**	**CV (%)**	**Mean ± S.E**	**Range**	**LSD (5%)**	**CV (%)**
Plant per plot	22.27 ± 0.75	23.67-21.00	1.74	4.1	22.13 ± 0.47	24.00-19.33	1.05	2.6
Early Blight	14.20 ± 3.70	18.00-11.70	8.53	31.9	17.53 ± 3.03	32.00-10.00	6.99	21.2
Late Blight	1.10 ± 1.37	2.50-0.50	3.16	152.6	0.77 ± 0.57	1.17-0.50	1.31	90.7
Incidence	4.60 ± 2.01	7.33-2.67	4.63	53.4	6.65 ± 1.98	8.93-1.50	4.56	36.4
Severity	3.17 ± 1.72	3.83-1.50	3.96	66.5	2.83 ± 1.94	4.17-1.17	4.48	83.9
Bacterial wilt	0.50 ± 0.0	0.50-0.50	*	0.0	1.97 ± 0.79	2.50-1.17	1.82	49.1
Fusarium wilt	0.50 ± 0.0	0.50-0.50	*	0.0	1.97 ± 0.90	2.50-1.83	2.08	56.1

APPENDIX 18: Mean, standard error, range, least significant difference and coefficient of variation were analysed for eleven quantitative traits.

	KNUST				CRI-KWADASO			
Traits	**Mean ± S.E**	**Range**	**LSD (5%)**	**CV (%)**	**Mean ± S.E**	**Range**	**LSD (5%)**	**CV (%)**
No. of plt harv.	79.10 ±11.31	103.3-59.7	26.09	17.5	71.1 ± 4.59	93.3-46.7	10.59	7.9
No. of MKT	269 ± 64.1	465-138	147.9	29.2	171.7 ± 21.34	277-92.7	49.22	15.2
No. of NMKT	95.3 ± 23.05	149.7-45.3	51.37	29.6	54.9 ± 14.11	112.3-27.7	32.54	31.5
MKT wt. (g)	377 ± 99.0	579-298	228.2	32.2	278.7 ± 31.22	465.4-157.6	72.00	13.7
Bulk MKT (kg)	23.8 ± 5.98	35.6-13.8	13.80	31.9	10.75 ± 2.26	21.40-5.72	5.22	25.8
Yield/ha	19.2 ± 6.86	29.7-11.5	15.29	43.9	8.96 ± 2.48	17.83-4.77	5.50	33.9
Brix	3.19 ± 0.19	3.40-2.96	0.43	7.1	3.36 ± 0.27	3.64-2.92	0.62	9.9
Shelf life	30.40 ± 0.0	40.00-22.00	*	0.0	30.40 ± 0.0	40.00-22.00	*	0.0
Fruit shape	3.40 ± 0.0	5.00-2.00	*	0.0	3.40 ± 0.0	5.00-2.00	*	0.0
TFB	1.83 ± 1.45	3.50-0.50	3.34	96.8	1.17 ± 1.16	2.50-0.50	2.66	121.2
BER	91.7 ± 23.78	149.3-41.3	54.84	31.8	52.8 ± 13.69	110.7-24.0	31.57	31.8

TFB: Tomato fruit borer MKT: Marketable

BER: Blossom end rot NMKT: Non-marketable

APPENDIX 19: Morphological characteristics, yield component and disease (ANOVA) table for tomato varieties at KNUST.

Plant per plot

Source	DF	SS	MS	VR	F pr.
REP	2	0.5333	0.2667	0.31	
Treatment	4	17.6000	4.4000	5.18	0.023
Residual	8	6.8000	0.8500		
Total	14	24.9333			

Average plant height

Source	DF	SS	MS	VR	F pr.
REP	2	0.90	0.45	0.01	
Treatment	4	540.42	135.10	2.16	0.164
Residual	8	500.31	62.54		
Total					

Average stem diameter

Source	DF	SS	MS	VR	F pr.
REP	2	16.709	8.355	2.31	
Treatment	4	14.718	3.680	1.02	0.454
Residual	8	28.946	3.618		
Total	14	60.373			

Average stem length

Source	DF	SS	MS	VR	F pr.
REP	2	7.2999	3.6499	5.86	
Treatment	4	58.8909	14.7227	23.64	<.001
Residual	8	4.9830	0.6229		
Total	14	71.1738			

Average inflorescence per plant

Source	DF	SS	MS	VR	F pr.
REP	2	35.344	17.672	3.27	
Treatment	4	5.669	1.417	0.26	0.894
Residual	8	43.243	5.405		
Total	14	84.256			

Average number of leaf under the1st inflorescence

Source	DF	SS	MS	VR	F pr.
REP	2	2.4813	1.2407	4.58	
Treatment	4	2.2867	0.5717	2.11	0.171
Residual	8	2.1653	0.2707		
Total	14	6.9333			

Growth habit

Source	DF	SS	MS	VR	F pr.
REP	2	0.00000	0.00000		
Treatment	4	3.60000	0.90000		
Residual	8	0.00000	0.00000		
Total	14	3.60000			

Number of flowers per inflorescence

Source	DF	SS	MS	VR	F pr.
REP	2	515.6	257.8	2.30	
Treatment	4	488.4	122.1	1.09	0.423
Residual	8	895.6	111.9		
Total	14	1899.5			

Number of days to 1st flowering

Source	DF	SS	MS	VR	F pr.
REP	2	2.533	1.267	0.60	
Treatment	4	34.400	8.600	4.10	0.043
Residual	8	16.800	2.100		
Total	14	53.733			

Number of days to 50% flowering

Source	DF	SS	MS	VR	F pr.
REP	2	2.5333	1.2667	2.45	
Treatment	4	27.0667	6.7667	13.10	0.001
Residual	8	4.1333	0.5167		
Total	14	33.7333			

Number of days to 100% flowering

Source	DF	SS	MS	VR	F pr.
REP	2	0.933	0.467	0.27	
Treatment	4	123.067	30.767	17.92	<.001
Residual	8	13.733	1.717		
Total	14	137.733			

Brix

Source	DF	SS	MS	VR	F pr.
Rep	2	0.18965	0.09483	1.86	
Treatment	4	0.53269	0.13317	2.61	0.116
Residual	8	0.40875	0.05109		
Total	14	1.13109			

Fruits shape

Source	DF	SS	MS	VR	F pr.
Rep	2	0.0000	0.0000		
Treatment	4	15.6000	3.9000		
Residual	8	0.0000	0.0000		
Total	14	15.6000			

Shelf Life

Source	DF	SS	MS	VR	F pr.
Rep	2	0.000	0.000		
Treatment	4	993.600	248.400		
Residual	8	0.000	0.000		
Total	14	993.600			

Number plant harvested

Source	DF	SS	MS	VR	F pr.
Rep	2	50.5	25.3	0.13	
Treatment	4	3719.1	929.8	4.84	0.028
Residual	8	1536.1	192.0		
Total	14	5305.7			

Number of marketable

Source	DF	SS	MS	VR	F pr.
Rep	2	55794.	27897.	4.52	
Treatment	4	271887.	67972.	11.02	0.002
Residual	8	49355.	6169.		
Total	14	377035.			

Number of non-marketable

Source	DF	SS	MS	VR	F pr.
Rep	2	108.1	54.1	0.18	
Treatment	4	13918.9	3479.7	11.65	0.002
Residual	8	2389.9	298.7		
Total	14	16416.9			

Marketable fruits weight (g)

Source	DF	SS	MS	VR	F pr.
Rep	2	9309.	4655.	0.32	
Treatment	4	156240.	39060.	2.66	0.112
Residual	8	117552.	14694.		
Total	14	283101.			

Bulk marketable fruits weight (kg).

Source	DF	SS	MS	VR	F pr.
Rep	2	588.07	294.03	5.48	
Treatment	4	1575.46	393.87	7.34	0.009
Residual	8	429.46	53.68		
Total	14	2592.99			

Yield/ha

Source	DF	SS	MS	VR	F pr.
REP	2	0.22	0.20	0.35	
Treatment	4	1092.86	273.21	3.87	0.038
Residual	8	706.42	70.64		
Total	14	1799.27			

Blossom End Rot

Source	DF	SS	MS	VR	F pr.
Rep	2	1120.9	560.5	0.66	
Treatment	4	31119.3	7779.8	9.17	0.004
Residual	8	6787.1	848.4		
Total	14	39027.3			

Bacterial wilt

Source	DF	SS	MS	VR	F pr.
Rep	2	0.	0.		
Treatment	4	0.	0.		
Residual	8	0.	0.		
Total	14	0.			

Early Blight

Source	DF	SS	MS	VR	F pr.
Rep	2	6.40	3.20	0.16	
Treatment	4	81.73	20.43	1.00	0.463
Residual	8	164.27	20.53		
Total	14	252.40			

Fusarium wilt

Source	DF	SS	MS	VR	F pr.
Rep	2	0.	0.		
Treatment	4	0.	0.		
Residual	8	0.	0.		
Total	14	0.			

Incidence (TYLCV)

Source	DF	SS	MS	VR	F pr.
Rep	2	126.400	63.200	10.48	
Treatment	4	44.933	11.233	1.86	0.211
Residual	8	48.267	6.033		
Total	14	219.600			

Late Blight

Source	DF	SS	MS	VR	F pr.
Rep	2	2.800	1.400	0.50	
Treatment	4	8.267	2.067	0.73	0.594
Residual	8	22.533	2.817		
Total	14	33.600			

Tomato Fruit Borer

Source	DF	SS	MS	VR	F pr.
Rep	2	12.133	6.067	1.93	
Treatment	4	22.000	5.500	1.75	0.233
Residual	8	25.200	3.150		
Total	14	59.333			

APPENDIX 20: Morphological characteristics, yield component and disease (ANOVA) table for tomato varieties at KWADASO

Plants per plot

Source	DF	SS	MS	VR	F pr.
REP	2	0.5333	0.2667	0.31	
Treatment	4	40.4000	10.1000	30.30	<.001
Residual	8	3.3333	0.3333		
Total	14	43.7333			

Average plant height

Source	DF	SS	MS	VR	F pr.
REP	2	9.32	4.66	0.25	
Treatment	4	1163.93	290.98	15.86	<.001
Residual	8	146.81	18.35		
Total	14	1320.05			

Average stem diameter

Source	DF	SS	MS	VR	F pr.
REP	2	2.464	1.232	1.20	
Treatment	4	3.444	0.861	0.84	0.538
Residual	8	8.222	1.028		
Total	14	14.131			

Average stem length

Source	DF	SS	MS	VR	F pr.
REP	2	0.563	0.282	0.10	
Treatment	4	73.694	18.423	6.59	0.012
Residual	8	22.375	2.797		
Total	14	96.632			

Average inflorescence per plant

Source	DF	SS	MS	VR	F pr.
REP	2	0.4853	0.2427	0.76	
Treatment	4	3.1507	0.7877	2.46	0.130
Residual	8	2.5613	0.3202		
Total	14	6.1973			

Average number of leaf under 1st inflorescence

Source	DF	SS	MS	VR	F pr.
REP	2	1.0973	0.5487	1.28	
Treatment	4	1.7573	0.4393	1.03	0.449
Residual	8	3.4227	0.4278		
Total	14	6.2773			

Average flower per inflorescence

Source	DF	SS	MS	VR	F pr.
REP	2	52.54	26.27	2.26	
Treatment	4	336.96	84.24	7.25	0.009
Residual	8	93.00	11.62		
Total	14	482.49			

Growth habit

Source	DF	SS	MS	VR	F pr.
REP	2	0.	0.		
Treatment	4	0.	0.		
Residual	8	0.	0.		
Total	14	0.			

Number of days to 1st flowering

Source	DF	SS	MS	VR	F pr.
REP	2	1.733	0.867	0.57	
Treatment	4	69.733	17.433	11.37	0.002
Residual	8	12.267	1.533		
Total	14	83.733			

Number of days to 50% flowering

Source	DF	SS	MS	VR	F pr.
REP	2	0.5333	0.2667	0.35	
Treatment	4	51.0667	12.7667	16.65	<.001
Residual	8	6.1333	0.7667		
Total	14	57.7333			

Number of days to 100% flowering

Source	DF	SS	MS	VR	F pr.
REP	2	6.533	3.267	2.58	
Treatment	4	85.067	21.267	16.79	<.001
Residual	8	10.133	1.267		
Total	14	101.733			

Plant size

Source	DF	SS	MS	VR	F pr.
REP	2	0.	0.		
Treatment	4	0.	0.		
Residual	8	0.	0.		
Total	14	0.			

Brix

Source	DF	SS	MS	VR	F pr.
Rep	2	0.0194	0.0097	0.09	
Treatment	4	1.5228	0.3807	3.46	0.064
Residual	8	0.8798	0.1100		
Total	14	2.4220			

Fruits shape

Source	DF	SS	MS	VR	F pr.
Rep	2	0.0000	0.0000		
Treatment	4	15.6000	3.9000		
Residual	8	0.0000	0.0000		
Total	14	15.6000			

Number of marketable

Source	DF	SS	MS	VR	F pr.
Rep	2	5760.5	2880.3	4.21	
Treatment	4	80974.0	20243.5	29.62	<.001
Residual	8	5466.8	683.3		
Total	14	92201.3			

Number of non-marketable

Source	DF	SS	MS	VR	F pr.
Rep	2	108.1	54.1	0.18	
Treatment	4	13918.9	3479.7	13.93	<.001
Residual	8	2498.0	249.8		
Total	14	16416.9			

Marketable weight (g)

Source	DF	SS	MS	VR	F pr.
Rep	2	2725.	1363.	0.93	
Treatment	4	153510.	38377.	26.24	<.001
Residual	8	11699.	1462.		
Total	14	167934.			

Bulk marketable weight (kg)

Source	DF	SS	MS	VR	F pr.
Rep	2	71.748	35.874	4.67	
Treatment	4	526.530	131.632	17.12	<.001
Residual	8	61.495	7.687		
Total	14	659.774			

Yield/ha

Source of variation	DF	SS	MS	VR	F pr.
REP	2	0.223	0.201	0.35	
Treatment	4	365.589	91.397	9.88	0.002
Residual	8	92.545	9.255		
Total	14	458.134			

Shelf life

Source	DF	SS	MS	VR	F pr.
Rep	2	0.000	0.000		
Treatment	4	993.600	248.400		
Residual	8	0.000	0.000		
Total	14	993.600			

Blossom End Rot

Source	DF	SS	MS	VR	F pr.
Rep	2	67.6	33.8	0.12	
Treatment	4	14289.7	3572.4	12.71	0.002
Residual	8	2249.1	281.1		
Total	14	16606.4			

Bacterial wilt

Source	DF	SS	MS	VR	F pr.
Rep	2	6.5333	3.2667	3.50	
Treatment	4	3.7333	0.9333	1.00	0.461
Residual	8	7.4667	0.9333		
Total	14	17.7333			

Early Blight

Source	DF	SS	MS	VR	F pr.
Rep	2	214.53	107.27	7.79	
Treatment	4	857.07	214.27	15.56	<.001
Residual	8	110.13	13.77		
Total	14	1181.73			

Fusarium wilt

Source	DF	SS	MS	VR	F pr.
Rep	2	6.933	3.467	2.85	
Treatment	4	1.067	0.267	0.22	0.920
Residual	8	9.733	1.217		
Total	14	17.733			

Incidence (TYLCV)

Source	DF	SS	MS	VR	F pr.
Rep	2	36.185	18.093	3.08	
Treatment	4	117.744	29.436	5.01	0.026
Residual	8	47.008	5.876		
Total	14	200.937			

Late Blight

Source	DF	SS	MS	VR	F pr.
Rep	2	0.1333	0.0667	0.14	
Treatment	4	0.9333	0.2333	0.48	0.749
Residual	8	3.8667	0.4833		
Total	14	4.9333			

Tomato Fruits Borer

Source	DF	SS	MS	VR	F pr.
Rep	2	13.333	6.667	3.33	
Treatment	4	8.000	2.000	1.00	0.461
Residual	8	16.000	2.000		
Total	14	37.333			