

**EFFECT OF GRAFTING ON BACTERIAL  
WILT DISEASE, YIELD AND QUALITY OF  
TOMATO (*Lycopersicon esculentum* Mill.) IN  
BURETI DISTRICT, KENYA**

**BY**

**JARED K. MUTAI**

**Reg. No. AGR/PGC/01/08**

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## **DEDICATION**

To my dear wife, Eglah, and daughters Janet, Cynthia and Angela, who convinced me that it was worth the effort, and with whom we thank God for everything.

**Jared Mutai**



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## ABSTRACT

Bacterial wilt disease is a major setback to greenhouse tomato production in Bureti district, Kenya. The objective of this study was to evaluate the effects of grafting rootstock on disease tolerance, growth, yield and quality of the popular greenhouse grown ‘Anna F1’ tomato variety. Four rootstocks were screened, namely two African eggplants (wild *Solanum aethiopicum* and a cultivated variety, DB3), and common local varieties of wild tomato (*Solanum lycopersici*) and goat apple (*Solanum aculeastrum*). Experimental transplants were established inside greenhouse planted in the same season, using RCBD experimental layout. Treatments involved planting of ‘Anna F1’ grafted on each rootstock with, and without inoculation with bacterial wilt disease, with similar planting of un-grafted ‘Anna F1’ plants as control treatments. Data was collected fortnightly on bacterial wilt incidence, disease scores and plant height (cm). Total yields and associated attributes were observed at the reproductive stage, while fruit quality parameters (fruit diameter, brix and pH) were examined at 90 days after transplanting. The data were analyzed for variance (ANOVA) using GLM of SAS computer program. Mean comparison was done using Tukey’s HSD range test. Wild *S. aethiopicum* and DB3 rootstocks completely controlled bacterial wilt disease, showing no symptoms on grafts. Wilt incidences were also significantly reduced to 22.2% and 44.4% among wild tomato and goat apple grafts, respectively, compared to up to 100% wilt incidence among non grafted ‘Anna F1’ plants. Wild *S. aethiopicum*, DB3, and wild tomato rootstocks also consistently improved scion yield, under disease inoculation, achieving up to 76.3%, 51.2%, and 38.1% yield increases, respectively. Despite reduced fruit quality, their wilt tolerance and yield stabilizing effects supported their recommendation to growers challenged by severe (wild *S. aethiopicum* and DB3), and moderate (wild tomato) bacterial wilt disease infestation. The results of this study provide farmers with an environmentally safe method for controlling bacterial wilt disease.

Key words: Rootstocks, ‘Anna F1’, bacterial wilt, grafting, greenhouse

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## LIST OF ACRONYMS AND ABBREVIATIONS

AVRDC	-	Asian Vegetable Research and Development Centre
HORTI	-	Horticultural Research and Training Institute (Tanzania)
DAT	-	Days after transplanting
MoA	-	Ministry of Agriculture
KARI	-	Kenya Agricultural Research Institute
PWP	-	Percentage wilted plants
PDI	-	Percentage disease incidence
OPV	-	Open pollinated variety
WAT	-	Weeks after transplanting
Wk	-	Week

# CHAPTER 1

## INTRODUCTION

### 1.1 Overview of tomato production in Kenya

Tomato (*Lycopersicon esculentum* Mill) originated in Peru/ Ecuador region of South America and is now cultivated widely throughout the world (van der Vossen *et al.*, 2004). It is grown for its fruits which are fleshy, red or yellow berries when ripe. The fruits are rich in vitamins A ( $\beta$ - carotene) and C and Lycopene (van der Vossen *et al.*, 2004; AVRDC, 2005). In the year 2010, Kenyan tomato production reached 590,000 metric tons, from about 19,000 ha, with average yield of 31 tons ha<sup>-1</sup>. This earned the subsector about Kshs 19 billion, representing 25% and 12% of earnings from vegetables and the entire horticulture subsector, respectively (MoA, 2010).

Tomato is one of the economically important crops affected by bacterial wilt disease, caused *Ralstonia solanacearum* (Yabuuchi et al 1995). This soil borne pathogen was first reported on solanaceous crops at the end of 19<sup>th</sup> century (Smith, 1896). Because of significant variation within the species, this pathogen is considered as a species complex, with variants identified into various groups, races, biovars, biotypes, sub-races and strains (Fegan and Prior, 2005). The pathogen affects more than 200 plant species. Some of the plants attacked by *R. solanacearum* include potato, eggplant, tobacco, bananas, potatoes and other cultivated tropical and subtropical crops and weeds (Bradbury, 1986; Hayward, 2000; OEPP/EPPO, 2004).

In Kenya, bacterial wilt disease has spread, mainly through latently infected tubers, to all the main potato growing areas in the highland regions, including Meru, Central Kenya, Molo, Timborua, UasinGishu, Keiyo, Marakwet, Transzoia, Bungoma, and Mount Elgon districts, where prevalence rates ranging from 71% to 90% have been reported (Ateka *et al.*, 2001; Olanya, 2002; Kwambai, 2008; Rotich, 2010). Bacterial wilt disease is particularly difficult to manage using conventional approaches due to its wide range of hosts and its ability to persevere through long crop rotations. The pathogen survives in the soil in the absence of host plants (Granada and Sequeira, 1983). The control of the disease

in greenhouse production is even more difficult since continuous production is inevitable (Oda, 1999).

Greenhouse tomato production is emerging as a popular system in the highland areas of Rift valley province. This is because all year round production in the open field is constrained by cold and wet conditions especially the long rains which favour fungal infection.

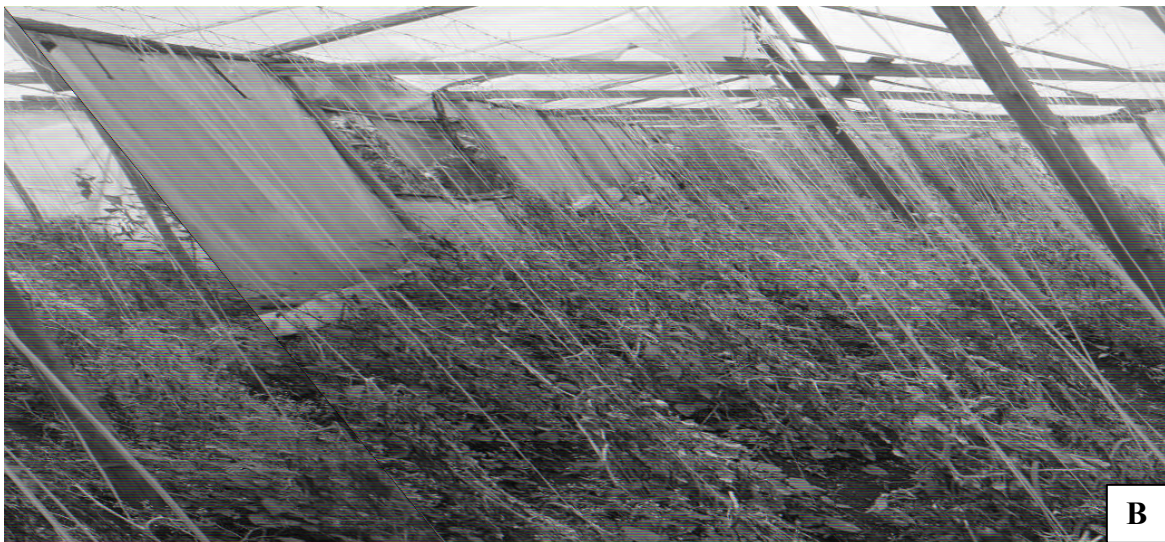
Grafting technique in tomato production has been used in reducing infection of plants by soil borne pathogens *Ralstonia solanacearum*, *Fusarium oxysporum* f.sp.*lycopersici*, *Verticillium dahliae*, *Pyrenochaeta lycopersici*, and *Meloidogyne spp*, (Besri, 2002; Miguel, 2004). In addition it has been used because of its advantages in promoting growth and increasing yield. It also confers tolerance to low temperature, enabling extension of growth period (Palada and Wu, 2005), salt tolerance (Rivero et al., 2003), and tolerance to floods (Black et al., 2003); and improves of the quality of fruits (Ruiz et al., 2007). However, the use of grafting technology to control bacterial wilt and improve yields has not been widely in Kenya.

## **1.2 Statement of the problem**

Currently greenhouse tomato production has been adopted by farmers in the cool, humid highland zones in Rift valley, Kenya. In these areas, cool night temperatures and high rainfall sometimes with hailstorms, limit outdoor production, in most part of the year (March – October), hence greenhouses system is necessary for all year round production (MoA, 2008). However, the incidence of bacterial wilt disease for greenhouse grown tomatoes is the major setback to year round production (Plate 1).

Due to the phasing out of methyl bromide which was previously the most effective soil sterilization fumigant, grafting with resistant rootstocks has emerged as the most practical alternative method for sustainable indoor production of tomatoes. Grafting has been reported to significantly decreased bacterial wilt incidence, while maintain a high fruit quality, even in severely infested soils in tomato production systems particularly in the

Mediterranean region and South East Asia (Williams *et al.*, 1991; Besri, 2002). Given the setbacks in the existing management options, there is therefore need to evaluate the suitability of grafting of popular greenhouse grown tomato varieties to potentially resistant rootstocks, which are locally available, as a management practice for controlling bacterial wilt disease, particularly in areas where the disease is endemic in Kenya.



**Plate1:** Bacterial wilt disease devastation on greenhouse tomato crops belonging to host farmers in Bureti district during the research programme, under their normal practice: at **(A):** Chesingoro site on ‘Anna F1’ variety, and at **(B):** Kapkatet site, on an indeterminate OPV variety, ‘Fortune maker.’

### **1.3 Justification**

Green house tomato production is a suitable technology enabling small holder farmers in cool and humid highland regions such as Bureti district, in Kenya, to produce tomato under protected conditions all year round. It is an emerging source of income which has a great potential in mitigation of poverty, food insecurity, unemployment in the district (MoA, 2008).

In this system, growers achieve higher productivity due to better control of environmental conditions, continuous cropping, use of high yielding varieties and high seeding rates. In addition, a fertile growing media with improved drainage, aeration and nutrient status is used. Growers also earn up to three fold more profits due to higher prices for greenhouse tomatoes produced during the long rains.

However, the greenhouse system is a setback where infection of the greenhouse with bacterial wilt disease occurs (Plate 1). This has led to farmers abandoning tomato production or using the greenhouses for production of other less profitable crops. The affected farmers ultimately fail to recoup the high investment cost of establishing the green houses. Moreover, greenhouse polythene covering material is costly and has a short lifespan of about 5 years.

Grafting using resistant rootstocks is a potential control option which has not yet been applied in this country. The purpose of the current study was to evaluate this technique as an alternative control option for controlling bacterial disease and enhancing green house tomato production in Bureti district, and may also be applied to other affected areas in Kenya.



## 1.4 Objectives

The overall objective was to improve the management of bacterial wilt disease in greenhouse grown tomato leading to high quality and yields.

The specific objectives were:

1. To compare local rootstock materials for tolerance to bacterial wilt disease
2. To determine the effect of grafting a selected greenhouse grown tomato variety to potentially tolerant rootstocks on bacterial wilt disease.
3. To evaluate the effect of grafting on growth, yield and quality of the selected greenhouse grown tomato variety.

## 1.5 Hypotheses

1.  $H_0$ : Local rootstock genotypes tested and tomato control variety are similarly affected by bacterial wilt disease  
 $H_1$ : Local rootstock genotypes tested are affected differently by bacterial wilt disease, in comparison to tomato control variety
2.  $H_0$ : Grafting has no effect in reducing bacterial wilt disease incidence on greenhouse grown tomato varieties grown under infested greenhouse condition  
 $H_1$ : Grafting reduces bacterial wilt disease incidence on greenhouse tomato varieties grown under infested greenhouse condition
3.  $H_0$ : Grafting has no effect on vegetative growth, yields and quality of the selected greenhouse tomato variety.  
 $H_1$ : Grafting affect vegetative growth, yields and quality of the selected greenhouse tomato variety.

## CHAPTER 2

### LITERATURE REVIEW

#### **2.1 Bacterial wilt disease in Tomato**

##### **2.1.1 Biology of Bacterial wilt disease**

Bacterial wilt disease is caused by the bacterium *Ralstonia solanacearum* which exist as a species complex with variants described variously as groups, races, biovars, phlotypes, sequevars, biotypes, sub-races and strains (Fegan and Prior, 2005). It affects over 200 cultivated crops and weed species. It is especially widespread in the tropics and subtropics, and to a lesser extent, in temperate areas where low temperature strains have been reported (OEPP/EPPO, 2004).

Tomatoes are highly susceptible to bacterial wilt caused by either race 1 or race 3. Occurrence of both races has been confirmed in Kenya (OEPP/EPPO, 2004). However race 3 (biovar 2) is likely to be more endemic in high elevations since it has a lower optimum temperature of 27°C, compared to race 1 which has a high optimum temperature of 35°C -37 °C. The disease is most severe under high soil moisture conditions (-0.5 to -1 bar) and temperatures of 24-35°C, overlapping with the same conditions required for optimum production of tomato under greenhouse conditions (Nesmith and Jenkins, 1985).

Bacterial wilt disease is caused after the pathogen invades and attaches to the xylem vessels where it highly multiplies blocking water conduction (Hayward, 1991; Denny, 2006). The initial symptoms are the flagging or flaccid appearance of 1 or 2 leaves on the plant. Thereafter, complete wilting follows within 4-7 days under favourable environmental conditions (OEPP/EPPO, 2004). Since other conditions such as *Fusarium* infection, root damage or water stress can cause similar wilt symptoms, it is necessary to include bacterial streaming ooze test as a field diagnostic symptomatic observation, although this may not be useful at early disease development stages (Mc Carter, 1991).

Laboratory identification can be done by isolation of the pathogen from diseased stems on Kelman's tetrazolium chloride agar medium to observe colonies of the bacteria. Colonies of *R. solanacearum* are observed after incubating for 36-48 hours at 30 °C. Virulent types appear as irregularly shaped round to oval, highly fluidal colonies, which are creamy white with pink centers; while non virulent mutants appear smaller, more round, and have uniformly dark red coloration (Denny and Hayward, 2001). Pathogenicity tests done by inoculating the isolates on indicator plants such as young tomato seedlings is also used to confirm the presence of the virulent pathogen. Additionally, morphological (Gram -ve bacterium with a single flagellum), and biochemical characteristics (Sudan black staining and Nile Blue test) can be used in laboratory identification (OEPP/EPPO, 2004).

The spread of the pathogen within a localized area occur through contaminated soil material, irrigation water or infected plant materials (Williams et al, 1991; Besri, 2002; AVRDC, 2005). Plant-to-plant infection can also occur when bacteria shed from infected roots move to roots of nearby healthy plant (Adebayo and Ekpo, 2006). Damage to roots caused by nematodes, handling during transplanting, or grafting without disinfecting between plants increases the rate of spread (Hayward, 1991; Priou, 1994). On the other hand the spread of the disease from one geographical area to another is known to occur through movement of material with latent infections such as potato tubers (Kwambai, 2008), geranium cuttings (Janse, 1996; Olanya, 2002), and tomato transplants (Mc Carter, 1991). Martins *et al.* (2002) concluded that true seeds are not a means of survival and dissemination of *Ralstonia solanacearum* in tomato.

The pathogen is well adapted to survive long periods of absence of host by lying dormant in soil, surviving in plant debris, irrigation or drainage water, or in weeds (Hayward, 1991). The survival of race 3 is dependent on host debris, latently infected potato tubers, and deep soil layers below 75 cm (Denny, 2006). It survives for 2 to 3 years in bare fallow pastures (Graham *et al.*, 1979). However, longer rotation periods of 5 to 7 years with non susceptible crops such as Maize have been recommended since the pathogen persists in micro lesions made in roots of these crops (Granada and Sequeira, 1983).

### 2.1.2 Significance of bacterial wilt disease

The disease distribution, incidence and severity vary from one region to another according to many factors such as the cultivars used, climatic conditions, soil type, cultural practices and diseases control methods used. In South East Asia, the estimated annual loss due to bacterial wilt disease in tomato range from 15% to 95%, worth about US\$ 25 to 158 million (Tsou and Shanmugasundaram, 1998). Up to 55% losses equivalent to US \$ 12 million annually have been reported in fresh market tomato in Taiwan (Hartman et al, 1991). In other tropical or subtropical countries in Asia and South American, Race 1 has been reported to be a serious constraint to tomato production and other solanaceous crops, but recent data on actual losses is limited (Ibrahim *et al.*, 2001; Lopez *et al.*, 2006).

In Africa, the presence of Rs3bv2 has been confirmed in 8 countries (Ethiopia, Malawi, Libya, Nigeria, Senegal, Sierra Leone, Somalia and Kenya). Although limited data on losses caused on tomato exist, infections by this race spread through potato production systems in tropical and subtropical environments can be severe, unlike in temperate regions where the race is limited by harsh winter temperatures (USDA, 2006; OEPP/EPPO, 2004). In Nigeria, up to 45% losses in tomatoes due to bacterial wilt disease have been reported annually, where about 38 % of farmers practice irrigation, which is the common means of the spread of this disease (Alamu, 2006).

In Kenya, upto 90% bacterial wilt incidence in potato farms have been reported in the major growing areas in the highland regions of Molo, Timborua, Mt. Elgon, Nanyuki and Meru (Olanya, 2002). In North Rift Valley counties of Trans Nzoia, Uasin Gishu, Keiyo and Marakwet, prevalence and incidence rates of 78.9% and 7%, respectively, have been reported (Kwambai, 2008). In Central Kenya, prevalence and incidence rates of 71% and 45.9%, respectively, were reported (Ateka *et al.*, 2001). In the Western region of Kenya, Rotich (2010) reported a bacterial wilt disease prevalence rate of 70% and an incidence rate of 6.9%, in Bungoma West district. In a survey of Western Kenya highland districts of Mount Elgon, Bungoma North and Bungoma West, bacterial wilt disease associated with the potato growing systems was found to be highest in the altitude range of 1800 m to 1999

m asl, where 7.2% disease incidence was reported (Rotich, 2010). While Nyangeri *et al.*, (1984) previously reported that the disease was more prevalent at lower altitudes because of higher temperature conditions favourable for the pathogen's survival and spread. However, the only pathogenic biovar (race 3 biovar 2A) found affecting potato, in Western highland region of Kenya is known to be adapted to lower temperature conditions (Van Elsas *et al.*, 2005), and has spread to higher altitude areas up to 2,399 m asl (Rotich, 2010). The disease trends in show an increasing geographical spread of the pathogen to higher altitude areas of Kenya.

Tomato green houses built on previous potato fields are likely to result in increased bacterial wilt infection rates on the tomato, due to disease development in latently infected tubers of previous potato crops, in response to more favourable greenhouse conditions. Nyangeri *et al.* (1984) showed that certified and healthy looking potato seed tubers with latent infection produced at altitudes of 1520-2120 m developed infection when planted at lower (warmer) altitudes.

### **2.1.3 Strategies of controlling bacterial wilt**

Cultural practices are currently the most popular means of controlling bacterial wilt disease (USDA, 2006; Priou *et al.*, 1994). These include using clean planting soils or media and practicing crop rotation with suitable crops like cereals, cucurbits (cucumber or Zucchini), alliums, brassicas and legumes. Other measures include control of alternate weeds and nematodes and use of uncontaminated irrigation water. Disease spread can also be minimized through quarantine or sanitation measures to avoid infections from infected fields. These include construction of diversion drains to control spread through movement or water runoff, and cleaning and decontamination of tools or shoes with water and calcium hypochlorite solution (USDA, 2006). Within infected fields, the effects of the disease can be partially reduced by liming, rouging of diseased plants, and by treating affected spots with wood ash (Kwambai, 2008).

Other major physical control options that have been applied as part of integrated control strategies consist of soil sterilization with steam before planting, use of soilless substrates,

and solarization (MBTOC, 2006; Dianez *et al.*, 2007; Page and Ritchie, 2007). Many variables influence their success and cost-effectiveness. Steam sterilization treatment should be carried out such that the coldest spot in the soil or substrate is maintained at 65°C to 80°C for half an hour (Fletcher, 1984; Pizano, 2007). When steam temperatures exceed 82 °C, problems of accumulation of soluble salts (particularly manganese and ammonium) and toxicity occurs, especially under high organic matter content. The economic viability of this method in greenhouse production can be improved by treating seed beds only, thereby reducing treatment costs by 40%. In this case, recontamination by untreated sections is prevented by using polythene for root separation (Fletcher, 1984).

There are few acceptable tolerant tomato cultivars documented such as Hawaii 7996, FL 7514, L285, and Tom 0126. Of these, Hawaii 7996 is the most promising, while the rest have only moderate resistance (USDA, 2006). However, Zarate *et al.*, (2006) has reported its susceptibility to a new pathogenic strain, T731, which confirmed that use of resistant tomato varieties may not give satisfactory results against hyper-virulent strains of the pathogen.

Fumigation using methyl bromide used to be the most effective means of bacterial wilt disease control (Besri, 2001). However, under the Copenhagen Amendment to the Montreal Protocol which came into effect from January 2005, this substance was added to the list of substances that deplete the ozone layer. It was subsequently banned in the EU and other member states who ratified the protocol (Batchelor, 2001; MBTOC, 2006). Biofumigants have been researched on, as alternatives to methyl bromide. Among them, thymol, which is the antibacterial fraction of essential oils, extracted from thyme and oregano medicinal plants, has been shown to suppress *R. solanacearum* and can control bacterial wilt (Ji *et al.*, 2005). Treatment of tomato field with thymol can be done practically through drip irrigation (Momol *et al.*, 2006). However, this substance is yet to be used commercially.

Other effective pre-plant chemical fumigants are metam sodium, 1, 3-dichloropropene + chloropicrin and dazomet (MBTOC, 2006). Although their effectiveness is more specific against nematodes, weeds and pathogenic soil fungi such as *Fusarium*, their use in

integrated control of bacterial wilt is important since positive interactions and synergy of *R. solanacereum* with these pathogens has been proved (USDA, 2006; MBOTC, 2006). The main disadvantage of chemical fumigation is the high cost of treatment. This can be reduced by 50 percent by combination with soil solarization for at least 15 days (Page and Ritchie, 2007). One microbial pesticide product  $0.1 \times 10^6$  cfu/g granular formulation of strain HY96-2 of *Paenibacillus polymyxa* is reported to have been registered for use in China, in 2004 (Li *et al.*, 2006). However, in comprehensive trials, the product only reduced bacterial incidence to 70-85% against 97% incidences in control (Li *et al.*, 2006).

Grafting has been considered as the most practical method of control in greenhouse production, where continuous cropping is practiced (Oda, 1999). It has been described as 'an old technology with a new technique', underlining the fact that although grafting of a vegetable like tomato is relatively new, it relies on an old principle (Rivard and Louws, 2006). Grafting has been recommended for use and adopted among growers for increased tolerance to diseases and other stresses; to increase crop vigour; as an IPM strategy; as an organic method; to promote food safety; and as a low-input, sustainable horticultural practice (Besri, 2002; Poffley, 2003; Palada and Ali, 2006). Combination of grafting with other alternatives has been used in some specific situations. For example, in the Mediterranean countries such as Greece and Morocco, it has been combined with alternative fumigants like 1,3-D or chloropicrin, solarization, or biofumigation because resistance of hybrid tomato rootstocks to *Verticillium dahliae* race 2., *Sclerotinia sclerotiorum* and *Clavibacter michiganense* break down under conditions of high salinity and high soil temperatures above 28-30°C (Besri, 2002)

Biofumigation, biosolarization, soilless cultivation, crop rotation, use of resistant varieties, and grafting are the main non-chemical alternatives that have been successfully harmonized into Integrated Crop Management (ICM) systems (MBOTC, 2006; Dianez *et al.* 2007). They are used to effectively control soil-borne diseases, weeds and root knot nematodes affecting vegetable production in Spain. However, for high-yielding vegetable varieties that are also highly susceptible to pathogens in the soil, grafting with rootstocks

highly resistant to various soil-borne pests proved to be the best solution (Bello *et al.*, 2007).

## **2.2 Grafting of Vegetable crops**

Grafting has been used in the horticultural industry for woody species, such as apples and grapes. The first use of grafting in vegetable crops was reported in Japan and Korea in the late 1920s whereby watermelon (*Citrullus lanatus*) was grafted onto gourd rootstock in order to diminish Fusarium wilt affecting watermelons. Grafting of eggplants started in the 1950s, followed by grafting of cucumber and tomato around 1960 and 1970, respectively (Edelstein, 2004).

Grafting has been increasingly used to control soil borne diseases in fruit bearing vegetables in Korea, Japan, and some Asian and European countries, where land use is very intensive and continuous cropping is common (USDA, 2006; Rivard and Louws, 2006). Oda (1995) reported that the proportion of area in Japan producing grafted watermelon, cucumber, melon, tomato and eggplant reached 59% of the total production area in 1990. In Greece, the ratio of the production area using grafted plants to the total production area, amounts to 90-100% for early cropping watermelons and 40-50% for melons under low tunnels, 2-3% for tomato and egg plants, and 5-10% for cucumbers (Traka-Mavrona *et al.* 2000). Grafted tomato has increased in Spain from less than one million plants in 1999 to about 45 million plants in 2003-2004. Grafted tomato is used in France on about 2,800 ha, to prevent problems such as corky root caused by *Pyrenochaeta lycopersici* (Dianez *et al.*, 2007; Besri, 2002).

In Italy, 10-12 million tomato plants are grafted annually. In Sardinia, the production of grafted tomato plants increased from almost nil in 1996 to about 1.7 million in 2003 (Miguel, 2004). The area under tomato production fumigated with methyl bromide in Sardinia reduced from 50% in 1992 to about 4% in 2004, due to agronomic changes which include the adoption of grafted plants and resistant varieties (Miguel, 2004). In Jordan, tomato grafting was introduced by the "methyl bromide phase out project" in 2002 and 1



ha of grafted tomato was planted (Besri, 2005). In Morocco, 20 million tomato plants are grafted, covering an area of 2000 ha equivalent to 50 % of the total plantations for export (Besri, 2002).

According to Oda (1999), inter-generic grafting is used in the production of many fruit-bearing vegetables. For example, cucumber (*Cucumis sativus* L.) is grafted on pumpkin (*Cucurbita spp.*), watermelon (*Citrullus lanatus*), on bottle gourd (*Lagenaria siceraria* Standl.), melon (*Cucumis melo* L.) on white gourd (*Benincasa hispida* Cogn.). Inter-specific grafting is generally applied to eggplant (*Solanum melongena* L.). Scarlet eggplant (*Solanum integrifolium* Poir) and *Solanum torvum* Swartz are popular rootstock for eggplant production (Rashid *et al.*, 2002). Researchers from the Asian Vegetable Research and Development Center recommended both inter-specific grafting tomato scions onto selected rootstocks of eggplants, and intra-specific grafting to resistant rootstocks to minimize problems caused by flooding and soil-borne diseases (AVRDC, 2003).

There are many grafting methods for different types of fruit-bearing vegetables: tomato plants and eggplants are mainly grafted by conventional cleft grafting (Lee, 1994;DPFIM, 2006; Dianez, *et al.*, 2007). Tube grafting has been developed for vegetable seedlings grown by plug culture. The survival ratio of grafted cucurbitaceae plants is higher if a tongue approach to grafting is used, especially for cucumber. This is because the root of the scion remains until the formation of the graft union. Slant-cut grafting has recently been developed for robotic grafting of watermelon and cucumber (Dianez *et al.*, 2007).

## **2.3 Grafting in tomato production**

### **2.3.1 The use of grafting technology in managing soil borne diseases**

The use of grafting to control the major soil borne diseases is increasing. Available data estimates use of grafted plants in selected countries is as follows: Japan 32%, Republic of Korea 5%, Greece 2–3%, Spain 10%, Morocco 25%, Netherlands 50% (Dianez *et al.*, 2007; MBTOC, 2006). Grafting has been used by growers to combine high-quality scions with suitable rootstock to obtain the benefits of disease resistance, stress tolerance, and

vigour (Palada and Wu, 2005; Rivard and Louws, 2006; Palada and Ali, 2006; Dianez *et al.*, 2007).

### 2.3.2 Tomato grafting rootstocks

Grafting of tomatoes on rootstocks from the same species, genus or family has been shown to be possible (Edelstein, 2004). The choice of specific rootstock is dependent on various factors, the most important being resistance to the most common soil borne pathogens in the area of use (Besri, 2002; Schippers, 2004), and compatibility with desired tomato scion cultivars (Ibrahim *et al.*, 2001; Lopes *et al.*, 2006) This is indicated by growth vigor and yield effects (Ibrahim *et al.* 2001; Besri, 2002; Kacjan-Marsic and Osvald 2004; Rashid *et al.*, 2002; MBTOC, 2006).

In the Mediterranean region, the most common soil borne pathogens reported are *Fusarium oxysporum f.sp.lycopersici* (races 1 and 2), *F. oxysporum f.sp.radicis lycopersici*, *Verticillium dahliae* (races1 and 2), *Pyrenochaeta lycopersici* , *Sclerotinia sclerotiorum*, *Didymella lycopersici* , *Clavibacter michiganense* subsp.*michiganense* and *Meloidogyne* spp (Besri, 2005). These pathogens are commonly controlled using the hybrid rootstock *L. esculentum* x *L. hirsutum*. However, where bacterial wilt disease (*Ralstoniasolanacearum*) is serious, resistance is provided by *S. torvum* and *S. aethiopicum*. In addition, these two rootstocks are resistant to *Meloidogyne* spp. (Besri, 2002; Schippers, 2004)

In the US, ‘Maxifort’ and ‘Beaufort’ rootstocks are widely used to enhance disease resistance to *Pyrenochaeta lycopersici* (Corky Root), most common species of nematodes, *Verticillium* sp, *Fusarium oxysporum* races 1 and 2, and *Fusarium oxysporum* fsp and *Radicis-lycopersici* (crown rot), and invigorates the plant (Rivard and Louws, 2006). Kacjan-Marsic and Osvald (2004) showed rootstock/scion variety compatibility between ‘Beaufort’ and ‘Monroe’, whereas ‘Beaufort’ was incompatible with ‘Belle’. In central Mexico, ‘Santa Clara’ tomato cultivar grafted on the rootstock of *Solanum lycocarpum* had no bacterial wilt incidence when planted in greenhouses infested with Rsr1bv1 but resulted in slower development , suggesting some scion/rootstock incompatibility.

In addition, H7996 rootstock was equally effective in controlling bacterial wilt and yielded normal plants (Lopes *et al.*, 2006).

There are reports on increase in tomato yields associated with control of bacterial wilt and other abiotic stresses such as flooding and typhoons by grafting of tomatoes to eggplant rootstocks. These range from 20-100% with *S. melongena* rootstock in Taiwan, Philippines, Lao PDR, Cambodia and Vietnam (Palada and Wu, 2005; Palada and Ali, 2006) to 145% increase with *S. torvum* rootstock in Bangladesh (Rashid *et al.*, 2002).

Ibrahim *et al* (2001) reported that grafting reduced bacterial wilt disease incidence and caused a narrow range of yield variation among the different rootstock/scion combinations, but found no effect of grafting on the number of trusses per plant, number of flowers per truss, number of flowers per plant, number of fruits per plant, percent fruit set rate and individual fruit weight. Also, there was a delay of 10 and 4 days in number of days to first flowering and number of days to first fruit harvesting, respectively, comparing *S. sisymbriifolium* tomato grafts and un-grafted control (BARI tomato-3). This was attributed to grafting shock, a common phenomena in grafted tomato. However, the delay did not affect final yield recorded at 65 tons ha<sup>-1</sup> and 49 tons ha<sup>-1</sup> for the same set of grafted and un-grafted tomatoes, respectively (Ibrahim *et al.*, 2001).

Based on experiences in the Mediterranean region and South East Asia, where soil borne diseases cause serious losses in tomato, the pea eggplant (*S. torvum*) and the African eggplant (*S. aethiopicum*) can be ranked among the most widely used rootstocks reportedly having well known resistance against *Ralstonia solanacereum* and *Melodogyne spp* (Ibrahim *et al.*, 2001; Besri, 2002; Rashid *et al.*, 2002; Schippers, 2004; MBTOC, 2006). *Solanum torvum* originated from central and south America and is now regarded as a pantropical weed (Schippers, 2004). In Africa, it is cultivated for consumption as a vegetable in Ghana, and used as a medicinal plant for preparation of antimicrobial concoctions in Gabon, Senegal, Sierra Leone, and Nigeria. The seeds require sunlight for germination. Flowering starts at 3-4 months and continues for its lifetime of about five years. Fruits are green when immature (eaten at this stage), then later mature into brownish yellow fruits containing many seeds (Schippers, 2004). The African eggplants also called

scarlet eggplants, or garden eggs comprise six interrelated species among which *Solanum aethiopicum*, *S. gillo* and *S. integrifolium* are the most horticulturally important indigenous edible species (Lister and Niaken, 1986; Blundel, 1987).

In Tanzania, African eggplants are cultivated for local market consumption in Tengeru rural, Ameru and Kilimanjaro districts (Abukutsa-Onyango, 2005). They are locally referred to as 'nyanya chungu' or 'bitter tomato', because their fruits which are orange or scarlet when ripe are bitter due to high concentrations of solanin. Seed is distributed by Alpha Seed Company. Growing involves transplanting of seedlings 4-6 weeks after sowing at a spacing of 1m x 1m and 10cm planting depth (Schippers, 2000; Abukutsa-Onyango, 2005).

### **2.3.3 Tomato grafting methods**

To ensure successful production of healthy grafted transplants, aspects such as rootstock selection, grafting technique, seeding dates, healing, and planting in the field must be carefully considered. Among the various techniques used for grafting in vegetable crops, cleft grafting and tube grafting are considered the most suitable in tomato grafting. In both techniques the scion is completely cut off from its roots and attached to the severed stem of the rootstock plant (Diáñez *et al.*, 2007).

With both cleft and tube grafting, the newly grafted plants must be protected from drying out until the graft union has healed. During graft healing, plants are kept in a tunnel or chamber, covered using a plastic (polythene) sheet inside to retain moisture and a shade material (shade nets or cheese cloth) outside to reduce light and, and then misted periodically during the day (Rivard and Louws, 2006) □ The required optimum conditions are 20–30°C, 80-90 percent relative humidity and low light. Plants must be ventilated about three days after grafting. Fusion occurs after 6-8 days, and then plants are moved to the areas for final adaptation. Transplanting can be done 14–21 days after grafting (Williams *et al.*, 2001)

### **2.3.3.1 Tube grafting in tomato**

Tube grafting is quicker and relatively simple because it only requires a single straight cut on both the root and shoot portions of the graft. This technique can also be used on very small seedlings, hence it is most recommended for plug seedling culture (Diáñez *et al.*, 2007). In this method, grafting must be carried out when the rootstock and scion seedlings have the same diameter. To achieve this, the rootstock is sown first and the scion, 2–7 days later. Grafting is done when the seedlings have two to four true leaves and the stems are 1.5 to 2 millimeters in diameter, at about 22–27 days after sowing (Rivard and Louws, 2006). The rootstock is cut at a slant angle (45–60°) up or under the cotyledons. The grafting clip is attached on the rootstock. The scion is cut in the same way, and then the two cut ends are placed in direct contact; the cut surfaces must make full contact (Rivard and Louws, 2006).

### **2.3.3.2 Cleft grafting**

Cleft (or wedge) grafting has been applied as a suitable method in inter-specific grafting of tomato cultivars to wild eggplant rootstocks (Williams *et al.*, 1991; Rashid *et al.*, 2002; Department of Primary Industry, Fisheries and Mines, 2006). In this method, both the scion and rootstock should be at four- to five true- leaf stage having a height of 20 cm and 3-5 mm thickness at the time of grafting. This is achieved by sowing of the wild eggplant rootstock into pots 2 - 3 weeks earlier than the tomatoes as they grow faster. During grafting, the rootstock is cut off at a height where it is the same thickness as the tomato, then leaves are removed, and the stem split down the centre to a depth of about 15 mm. Next, a tomato scion is cut off below the growing tip, and most of the large leaves trimmed back. The base of the tomato cutting is then cut down each side into a wedge shape, and inserted into the rootstock, ensuring good contact. A peg or a clip is placed on the graft union to hold it in place. The graft is then covered with a plastic bag to maintain humidity, or placed inside a shaded, humid healing chamber, until the graft forms a callus, and then peg or clip is removed. The grafted plants are subsequently mulched and staked to avoid

contact of the grafted area with soil, which can infect the plants with bacterial wilt disease (Williams *et al.*, 1991; Department of Primary Industry, Fisheries and Mines, 2006).

### **3.3.4 Grafting success rates**

High grafting success rates can be achieved in tomato particularly using the cleft grafting technique. In grafting of tomato 'Prelane' F1 onto eggplant rootstock using different techniques, the highest success ratio was obtained from cleft grafting with 83.3 %, whip and tongue grafting at 69.7% and 43.7% in lateral perforation techniques (Vuruksan and Yanmaz, 1990).

Kacjan-Marsic and Osvarld (2004) compared the success of cleft grafting and tube grafting methods on tomato cultivar 'Monroe' grafted on Beaufort and PG3 rootstocks and observed the highest survival rates (100%) when 'Monroe' was cleft grafted on either rootstock. However, tube grafting 'Monroe' on 'PG3' rootstock, had the lowest survival rates (79%).

Ibrahim *et al.*, (2001) observed 78-92 % grafting success using 35 day old tomato scions cleft grafted on 50 day old wild solanum rootstocks. They concluded that grafting success rate was high irrespective of the type of rootstock used. Farmers trained on the tomato grafting achieved more than 95% transplanting success rates in Bangladesh, where the technique has been successfully evaluated to be the most cost effective strategy against bacterial wilt disease and heat stress, flooding, and typhoons (Hanoi Seed Company, 2005)

## **2.3.5 Benefits of tomato grafting**

### **2.3.5.1 Disease resistance**

Grafting has been demonstrated to be effective against a variety of soil-borne fungal, bacterial, viral, and nematode diseases (Williams *et al.*, 1991; Besri, 2005; Rivard and Louws, 2006). It has been used to eliminate verticillium and fusarium wilt in tomato and cucurbit production systems in Japan, Korea, and Greece. In New Zealand, it has been used to reduce levels of corky root rot (Rivard and Louws, 2006). In Morocco and Greece,

grafting is used to control root-knot nematodes (*Meloidogyne* species) in both tomatoes and cucurbits. Grafted plants are used instead of methyl bromide to manage soil-borne diseases in Morocco and Greece (Oda 1999; Besri, 2002; MBTOC, 2006).

Grafting has been essential in Asian horticultural production for eliminating bacterial wilt incidence in tomatoes and other solanaceous crops. It is reported that in the South-east Asian kingdom of Brunei, where bacterial wilt incidence is so high that tomatoes cannot be planted unless the soil is sterilized or resistant rootstocks are used (Peregrine and Binahmad, 1982; Williams *et al.*, 1991). In Bangladesh, bacterial wilt disease is common in non-flooded highlands where solanaceous vegetables are grown continuously without crop rotation, with 100% losses sometimes reported in Kitchen gardens (Ibrahim *et al.*, 2001). In India, wilt-resistant CRA 66 rootstocks were used to reduce bacterial wilt in tomatoes. By the end of the season, none of the control plants had survived while 100 percent of the grafted plants continued to produce. Furthermore, the yield of the tomatoes with resistant rootstocks was four times that of the non-grafted susceptible plants (Tikoo *et al.*, 1979).

Grafting with salt-tolerant rootstocks has been used in areas affected by high salinity (Rivero *et al.*, 2003). Worldwide, salinity is estimated to cover more than one-third of all irrigated areas, causing yield losses. Grafting has also been used to reduce the negative effects of excess moisture in the soil (Black *et al.*, 2003). Asian Vegetable Research and Development Center reported that tomatoes are difficult to grow during the hot-wet season, because the effects of flooding, water-logged soils, and high temperatures combined with diseases leads to significant reduction in yields (Black *et al.*, 2003). AVRDC recommends grafting tomato scions onto selected rootstocks of eggplant or tomato to minimize these effects.

Grafted plants have also been shown to have effective tolerance to soil temperature extremes, and hence allow the growing season to be extended in either direction. This helps growers to raise the selling price of their produce hence improve their incomes (Palada and Wu, 2005; Rivard and Louws, 2006).

### **2.3.5.2 Increased productivity**

Grafting *per se* has been shown to increase yields even without the presence of disease or abiotic stressors. For example, the Maxifort and Beaufort rootstocks used in greenhouse tomato production systems has been utilized as a vigorous rootstock and increase fruit yields, even where little disease pressure is evident (Lee, 1994, Rivard and Louws , 2006). Such vigorous rootstock varieties are known to have vigorous root systems which enhance water and nutrient uptake in grafted plants, leading to increased productivity (Lee, 1994, Rivard and Louws , 2006; Leonardi and Giuffrida, 2008).Leonardi and Giuffrida (2008) reported positive nutrient uptake interactions in inter-specific eggplant rootstock / tomato graft combinations, and reported 100 – 300% greater uptake of calcium, phosphorous, and sulphur compared to intra-specific tomato graft combinations. In Morocco, growers have taken advantage of increased productivity associated with vigorous rootstocks to reduce planting densities by 50% (from 18,000 - 20,000/ha plants to 9,000- 10,000 plants/ha), hence reducing the cost of the plants (Besri, 2002; MBTOC, 2006).

### **2.4 Small holder greenhouse tomato production system in Bureti district**

Green house tomato production is emerging as an important technology being adopted by farmers in Bureti district. This technology is also gaining prominence particularly in the highland areas in Rift valley province, including the wider Uasin Gishu, Keiyo, Nandi and Kericho districts. These areas experience cool, humid climatic conditions limiting outdoor cultivation of tomato during the long rains season. This system has been adopted by more than 500 small scale farmers in this region. The commonest greenhouses used by small scale farmers are simple plastic units measuring 15 m x 6m, with a capacity of about 600 plants (MoA, 2008).

Although there are no precise data on the small holder greenhouse tomato production system, it is known, based on observation and personal communication with farmers and extension officers from the study area, to involve high investment costs. The cost of UV



treated greenhouse polythene (Lifespan of 5 years), and establishment of planting beds substantially contribute to high cost of greenhouse establishment, averaging about USD 1000 per unit.

Greenhouse varieties planted by growers include Alletta F1, 'Anna F1', Eden F1, and Monset F1. The preferred varieties have indeterminate growth (utilizes available vertical space inside greenhouse); high yields; long harvesting period; and long post harvest shelf life demanded in the market.

Planting is usually done on double dug beds filled with growing media mixed using rotten manure, saw dust, and aggregates (charcoal or gravel chips) in order to improve nutrition, aeration, and drainage properties to support high density plantings. Re- addition of manure to enrich the growing media is the main medium amendment practice carried out between crops.

Continuous cropping with tomato after tomato is a common feature. Crop rotation is not practiced due to lack of other alternative profitable crops and need to make maximum use of the greenhouse to recoup investment costs within the limited lifespan of the greenhouse polythene sheet. The cropping cycle is usually 1 crop every 9 months (comprising 1 month nursery stage; 6 months cropping; and 2 months discard of old crop and remaking of beds for next replanting). The farmers attempt to produce at least 3 crops in 2 years continuously if no limiting soil-borne disease is observed.

Bacterial wilt disease is an important constraint affecting green house tomatoes as well as other solanaceous crops (potato, peppers/ chillies) grown in the highland region of Rift valley. Rouging is the main control option in greenhouses infected with bacterial wilt recommended to farmers.

## CHAPTER 3

### MATERIALS AND METHODS

#### 3.1 Screening of rootstocks for bacterial wilt disease tolerance

##### 3.1.1 Rootstocks planting materials used in the study

Seeds of four varieties of solanum species selected for bacterial wilt disease tolerance screening were obtained and used to raise rootstock seedlings for the study: Wild African eggplant (*Solanum aethiopicum*); cultivated African eggplant variety DB3 (*S. aethiopicum*); wild cherry tomato variety ‘chelolo’ (*Solanum lycopersicum*) and goat apple (*S. aculeastrum*). The wild and cultivated African eggplant seeds were obtained from the National gene bank, Nairobi, Kenya and Tanzanian National Horticultural Research and Training Institute (HORTI), Arusha, respectively. Wild cherry tomato and goat apple seeds were collected locally from farmers. One popular greenhouse tomato variety (‘Anna F1’) was selected as a standard when self rooted. Certified seeds of this variety, which originated from De Ruiters Seed Company, The Netherlands, were obtained from local dealers.

##### 3.1.2 Establishment of experimental rootstock plants

Seeds of each rootstock were first sown in seed trays to germinate, on sterilized loamy soil, then pricked at into small polythene tubes of size 8cm x 20cm, filled with the same loamy substrate, one week after germination. The tomato (standard) variety seeds were similarly sown, but 3 weeks later, in order to synchronize the growth stage at transplanting, with that of the rootstocks, which take longer to germinate and have slower growth rate. The resulting seedlings were raised inside greenhouse, under optimum moisture and nutrient levels up to transplanting stage. Transplanting was then done into polythene pots of size 20cm (base) x 15cm (width) x 35cm (height) each filled with about 10 Kg of media consisting of loamy soil, decomposed farm yard manure, and coarse sand in the ratio 3:1:1. The potting media was previously steam sterilized at 65 – 75°C for 30 minutes, according

to Fletcher (1984). The potted transplants were established inside 2 selected farmer greenhouses, at different locations, in the same season.

### **3.1.3 Experimental design and layout**

Potted test plants were arranged inside each greenhouse into plots with each six plants, following RCBD experimental layout having three blocks each consisting 10 plots: five plots of each kind (wild *S. aethiopicum*, DB3, wild cherry tomato, goat apple, and ‘Anna F1’ tomato cultivar (standard check)) given bacterial wilt inoculation treatments; and five plots of each kind, without bacterial wilt disease inoculation.

### **3.1.4 Inoculum preparation and inoculation**

A standard *Ralstonia solanaceaurum* inoculum suspension was prepared in the National Agricultural Research Laboratory, Kenya Agricultural Research Institute as follows: Naturally infected tomato plants were collected from previously existing crops in farmer greenhouses in the study area and chopped into two inch pieces which were then cut diagonally and covered with 1 inch of sterile water in a beaker, for 24 hrs. The milky bacterial exudates that appeared were collected using a sterile platinum loop and streaked on a tetrazolium chloride medium (Kelman, 1954), and incubated at 30°C for 48 hrs. Typical round to oval fluidal colonies of *R.solanaceaurum* were then re-isolated and propagated for a further 48 hours by incubating at 30 °C in culture plates containing fresh Kelman’s tetrazolium chloride agar, in order to obtain adequate pure cultures. Bacterial masses were subsequently harvested and suspended in sterilized distilled water then diluted to  $1.0 \times 10^6$  CFU, using optical density measurement. The resulting suspension was then transferred to experimental sites and used to inoculate the test plants within 24 hrs.

Disease inoculation treatment was done were done 3 weeks after transplanting (WAT), using the root inoculation technique. Secondary roots on one side of the experimental seedlings were exposed and cut to 2 -3 cm from the tap root by passing a sterile knife to a depth of 4-6 cm, to cause sufficient damage to facilitate infection. A 50 ml suspension containing about  $10^6$  CFU of *Ralstonia solanaceaurum* were poured over the injured roots. The exposed roots were then covered back with the media. Roots of non-inoculated

experimental plants used as control were similarly injured, but 50ml of plain sterile water poured. All the plants were then watered frequently to maintain favourable soil moisture conditions within the root zone, to ensure survival of the pathogen, hence disease development. The inoculum levels were maintained by making a boosting inoculation treatment, 1 month after the initial inoculation.

### **3.1.5 Greenhouse growing conditions**

The potted experimental plants were established at a spacing of 40cm x 30 cm inside the greenhouses. D.A.P 20gm fertilizer (18-48-0) per plant were added and mixed well with the potted media and adequate watering done at planting. Each plant was top-dressed with 20gm C.A.N fertilizer (26%N) per plant in 2 splits each 10gm per plant, at the 4<sup>th</sup> and 8<sup>th</sup> week after transplanting, respectively. Only tomato plants were supported upright. All the plants were mulched and other husbandry practices such as pest and disease control, weed control, irrigation, and de-suckering were done regularly as required.

### **3.1.6 Disease incidence and severity assessment**

After transplants were established, bacterial wilt disease (incidence and severity) and plant growth measurements were made at fortnightly intervals, until 18 weeks after transplanting. Bacterial wilt disease symptoms on each plant were scored using a scale of 0-5, modified from Truong (2007): 0 = No wilt; 1= wilt on 1 or 2 leaves; 2= wilt on about half (50%) of the leaves; 3 = Wilt on all except 1 or 2 leaves; 4 = wilt on all leaves; 5 = Death or collapse of whole plant.

From the wilt score data, Bacterial wilt disease incidence was determined as the percentage of wilted plants (PWP) in each plot, calculated using the formula according to Rotich (2010):

$$PWP = (N_w/N_T) \times 100$$

Where  $N_w$  = Number of wilted plants (score 1-5) and  $N_T$  = Total number of inoculated plants

Disease severity was determined as percentage disease index (PDI) calculated using the formula according to Truong (2007):

$$\text{PDI} = [(N_0 \times 0 + N_1 \times 1 + N_2 \times 2 + N_3 \times 3 + N_4 \times 4 + N_5 \times 5) / (N_T \times 5)] \times 100$$

Where  $N_0$  to  $N_5$  = Number of plants at each score; and  $N_T$  = Total number of inoculated plants.

### **3.1.7 Growth measurement**

Growth was assessed through measuring plant height of all plants in each plot, from the soil level to the end of the main (longest) shoot tip, using a measuring tape, at fortnightly intervals. The absolute plant height measurements (cm) were made at fortnightly intervals, beginning at transplanting stage up to 18 WAT. These measurements were then used to compare growth effects between bacterial wilt disease inoculated and non-inoculated plants of the same rootstock variety.

## **3.2 Determination of the effects of grafting on bacterial wilt disease**

### **3.2.1 Establishment of grafted experimental plants**

Seeds of four varieties of solanum species were selected from the first study: Wild African eggplant (*Solanum aethiopicum*); cultivated African eggplant variety DB3 (*S. aethiopicum*); wild cherry tomato variety ‘chelolo’ (*Lycopersicon esculentu*); and goat apple (*S. aculeastrum*) were used to raise rootstocks for grafts. In addition, some of the certified seeds of ‘Anna F1’ tomato variety in study 1 were used to raise scion seedlings for grafts.

Rootstock seedlings of various varieties of solanum were raised as described in section 3.1. When scion seedlings attained 20- 25 cm, they were selected and cleft (wedge) grafted to 20 cm high rootstock seedlings cut back to 10 – 15 cm grafting points having diameter of 3-5mm. The graft insertions were strapped with a small section of plastic straw, tied together tightly using a polythene film strip, then held at 20<sup>0</sup>C-30<sup>0</sup>C, under 80-90% relative

humidity, in a healing chamber constructed using layers of polythene covering and shade net materials (Oda,1999; Black *et al.*, 2003), for one week. The grafts were further raised for two weeks inside greenhouse, in order to acclimatize and attain normal seedlings growth vigor before transplanting, according to Williams *et al.* (1991).

Transplanting of grafts inside farmer's greenhouses was done concurrently with transplanting of rootstocks established in the first study. The same procedure and substrate, as for rootstocks, was used in the establishment of grafts as outlined in section 3.1.

### **3.2.2 Experimental design and layout**

The four types of potted grafts were arranged inside the farmer greenhouses using RCBD with three blocks each having eight plots, and six plants per plot. The eight plots in each block comprised of two plots for each graft type: one plot inoculated with bacterial wilt disease; and the other non-inoculated (control). Since the experiments on evaluation of disease tolerance both among rootstock pairs (study 1) and grafts (study 2) were run concurrently, the two plots of un-grafted 'Anna F1' seedlings already included in each block (bacterial wilt disease inoculated and non-inoculated) in the first study, also served as standard-check for the second study.

Inoculation treatments on grafts were done concurrently with rootstock inoculation treatments, at 3 WAT growth stage, using the root inoculation technique, following the same procedure for rootstocks as outlined in section 3.1

The potted grafts were established and raised inside greenhouse under the same growing conditions as for rootstocks, with respect to spacing, fertilizer application, and other crop husbandry practices, as described in section 3.1. In addition, all the plants were mulched with dry grass and supported upright to avoid contamination of grafted plants at the graft union with soil borne pathogens.

Bacterial wilt disease incidence and severity were assessed and PWP and PDI calculated as outlined in section 3.1

### **3.3 Determination of the effect of grafting on growth, yield and quality of tomato**

### 3.3.1 Establishment of experimental plants

The effect of grafting on tomato growth, fruiting, total yield and fruit qualities were examined in the same sets of both bacterial wilt disease inoculated , and non-inoculated grafted test plants established in study 2, which were simultaneously evaluated for disease tolerance, in the two experimental sites. In the third study, plant growth, fruit yield and quality parameters were observed fortnightly as outlined below:

### 3.3.2 Assessment of growth parameters

Growth was determined by taking plant height measurements of all plants in each plot. A measuring tape was used to determine the length of the main stems from base to apex. The effects of grafting on growth of grafts were then compared using the following parameters:

**Actual Plant height:** This parameter was used to compare achieved plant height at fortnightly intervals, from initial and subsequent days up to termination of the experiment.

**Plant growth rate (PGR):** This parameter was used to compare the change in plant height, at every fortnightly interval, and was determined as follows:

$$\text{PGR (cm/fortnight)} = (H_2 - H_1)$$

Where: PGR = plant growth rate, in cm per fortnight

$H_1$  = Initial plant height (cm) measured at the beginning of each observation interval.

$H_2$  = Plant height (cm) measured at fortnight intervals.

### 3.3.3 Assessment of yield parameters

When the plants reached bearing stage, the point of initial harvest in each plot was observed, being the number of days taken for first cluster fruits to reach colour break maturity stage. Thereafter, ripe fruits (at least at colour break stage of ripening) continued to be harvested from each plot at intervals of 7 to 14 days. The picked fruits were counted and weighed using a weighing scale, and the cumulative results of all harvests during the experimental period were averaged to determine the yields parameters: number of

fruits/plant and weight of fruits (g/ plant) for each plot. Cluster yields were similarly determined by averaging cumulative fruit set counts and number of clusters set per plant.

### **3.3.4 Assessment of fruit quality parameters**

Fruit quality measurements were done on fruits harvested from each plot at color break stage of ripening, 90 days after transplanting (DAT), which was considered the mid harvesting point. The harvested fruits were pooled together regardless of cluster level. Fruit diameter was determined by randomly selecting 10 fruits per plot, harvested at 90 DAT, and diameter measurements made using a pair of vernier calipers.

Internal fruit quality (pH and Brix) analyses were done at the Kenya Sugar Research Foundation laboratory. Each fruit sample of approximately 500g was washed, cut, and pound using pestle and mortar, then about 50ml of the resulting pulp was decanted into a small beaker. The pH of the pulp was measured directly using an electronic pH meter.

Each of the decanted pulp samples were filtered through a filter paper to obtain about 5ml of clear filtrate, which were subsequently used for Brix measurement using an Automatic digital refractometer (Index instruments, UK, Great Britain).

### **3.4 Data analysis**

The data obtained from each study were subjected to analysis of variance (ANOVA), using the SAS computer program's GLM procedure (SAS 9.1, SAS institute, Cary, NC, USA) to examine significant effects of rootstock types, grafting and disease inoculation on the parameters observed on bacterial wilt disease incidence, plant growth, yield and quality. Means comparison was performed using Tukey's HSD range test, at alpha value,  $\alpha = 0.05$



## CHAPTER 4

### RESULTS

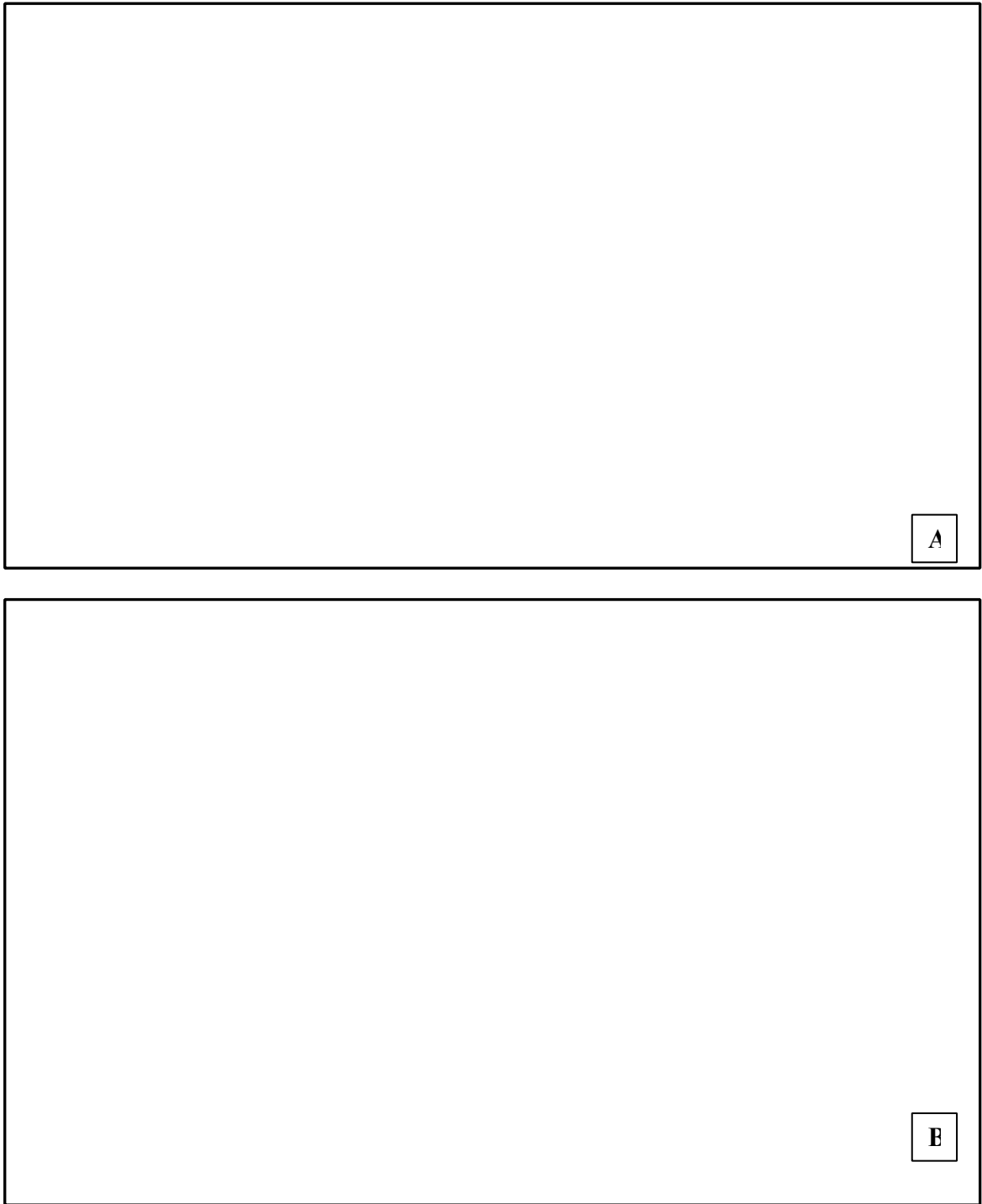
#### 4.1 Tolerance of rootstocks to bacterial wilt disease tolerance

##### 4.1.1 Disease incidence and severity effects on rootstocks

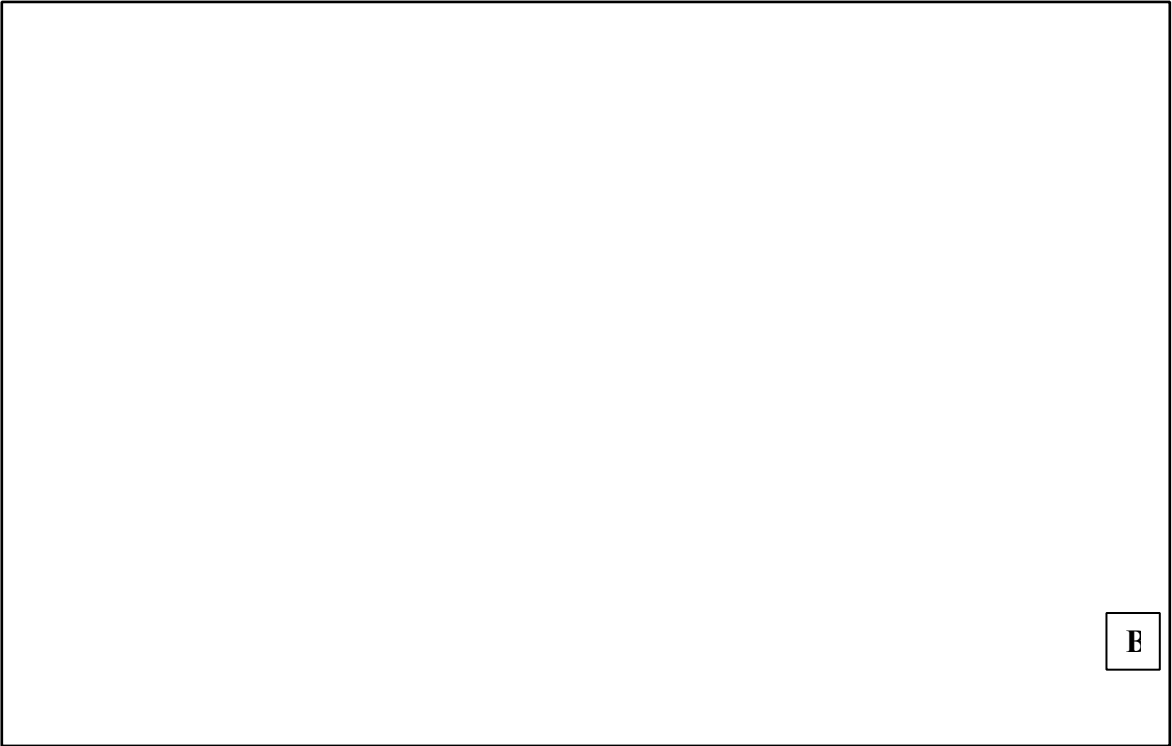
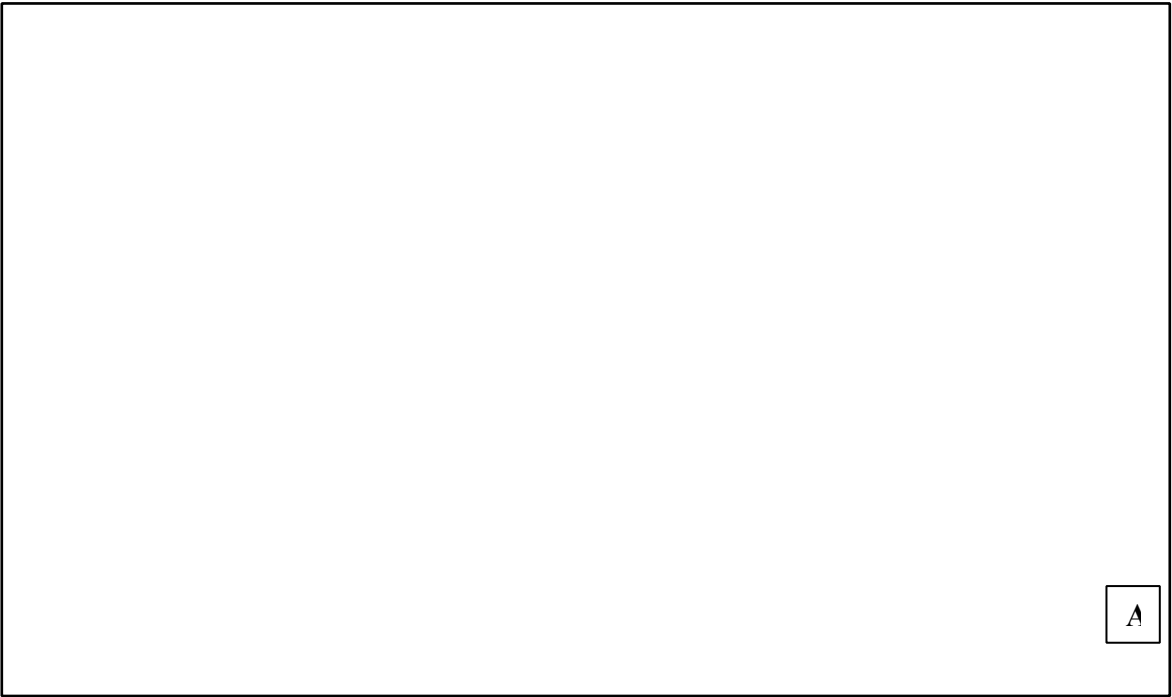
Bacterial wilt incidences were observed among inoculated rootstocks from 14 weeks after transplanting (WAT) across the two study sites. This was approximately 10 weeks and 6 weeks after initial and repeat inoculation with bacterial wilt disease inoculum, respectively. When bacterial exudates from wilted experimental rootstock plants were re-isolated in Kelman's tetrazolium chloride agar medium, round to oval fluidal bacterial colonies with red centered pigmentation, typical of *R. solanacearum* were observed, confirming the cause of wilting to be due to bacterial wilt disease.

Significant differences were observed with respect to disease incidence, expressed as Percentage wilted plants (PWP), from 14 WAT, in both sites (Figure 1; Appendix I-1). PWP reached 100% at 18 WAT in un-grafted Anna F1 control plants at Chesingoro site. Goat apple recorded a maximum PWP value of 44%, at Chesingoro site (Figure 1A), while the maximum PWP value for wild was 22%, at Kapkatet site (Figure 1B). The PWP values for the two rootstocks were significantly lower compared to those of Anna F1 control. There was no incidence of wilt in both wild *Solanum aethiopicum* and DB3 rootstocks tested, across both sites (Figure 1; Appendix I-1).

Percent disease severity index (PDI) was higher on goat apple (maximum PDI value of 21.1%) than on wild tomato (maximum PDI value of 14.4%), at Kapkatet site, but this difference was not statistically significant ( $p>0.05$ ) (Figure 2B; Appendix I-1). However, both rootstocks exhibited significantly ( $p<0.05$ ) lower disease severity compared to the un-grafted Anna F1 control, which recorded the highest PDI values, reaching 82.7%, at 18 WAT, at Chesingoro site (Figure 2A; Appendix I-1).



**Figure 1:** Disease incidence, observed as PWP, among inoculated rootstocks in **(A)** Chesingoro site, and **(B)** Kapkatet site.

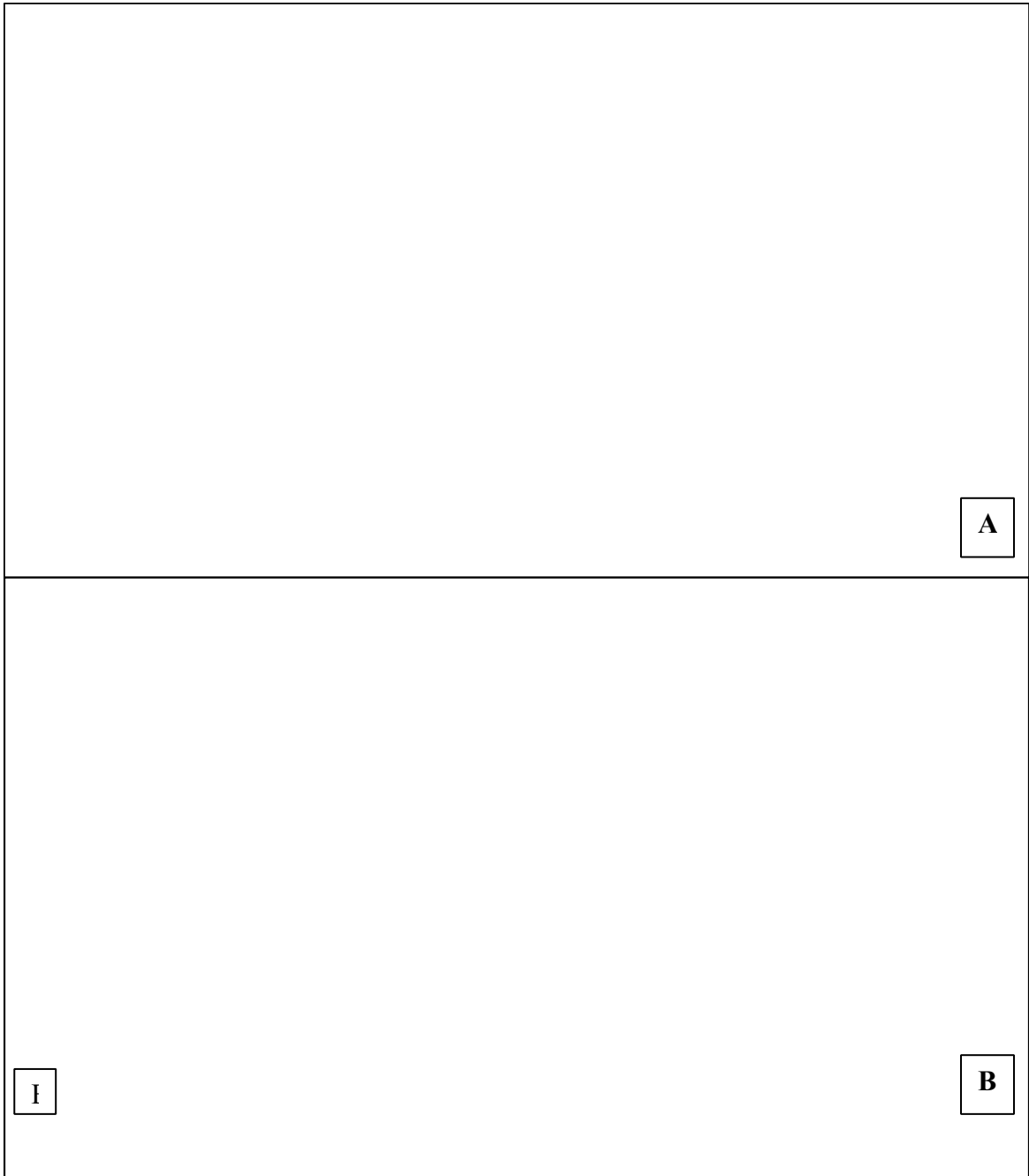


**Figure 2:** Disease severity observed as PDI, among inoculated rootstocks, during 14-18 WAT, at Chesingoro site (A), and at Kapkatet site (B).

#### 4.1.2 Effects bacterial wilt on growth of rootstocks

Disease inoculation elicited growth suppression among all rootstocks, and also on un-grafted Anna F1 control plants in comparison to their non-inoculated counterparts. However, this was least evident in wild *S. aethiopicum*, which recorded a height reduction of 1.5 cm, at Chesingoro site (Figure 3A; Appendix I-3). Under bacterial wilt disease inoculation, plant heights for DB3 rootstock were reduced (by 3.8cm) compared to *S. aethiopicum*, despite absence of visible wilting symptoms on both of them during the experimental period (Figure 3; Appendix I-3). At Kapkatet site, the height of inoculated DB3 rootstocks were decreased by up to 13.2 cm, while the height of wild *S. aethiopicum* was reduced by only 0.3 cm, in comparison to corresponding non-inoculated sets of plants (Figure3B; Appendix I-3).

Wild tomato and goat apple rootstocks, showed both wilt symptoms and growth suppression following inoculation. However, growth suppression was higher in goat apple than in the wild tomato by 5.7 cm at Chesingoro site, at 18 WAT (Figure 3; Appendix I-3). At Kapkatet site, growth of goat apple was also more suppressed than in the wild tomato by 10 cm, at 18 WAT (Figure 3; Appendix I-3). Disease inoculation also resulted in reduced heights of the Anna F1 tomato control plants, by 14.9 cm and 14.4 cm at Chesingoro and Kapkatet sites, respectively. However, the decreased height among inoculated plants of both rootstocks and Anna F1 tomato control plants were not statistically significant ( $p \geq 0.05$ ), in comparison to non-inoculated set of plants of each type, at 18 WAT (Figure3; Appendix I-3).

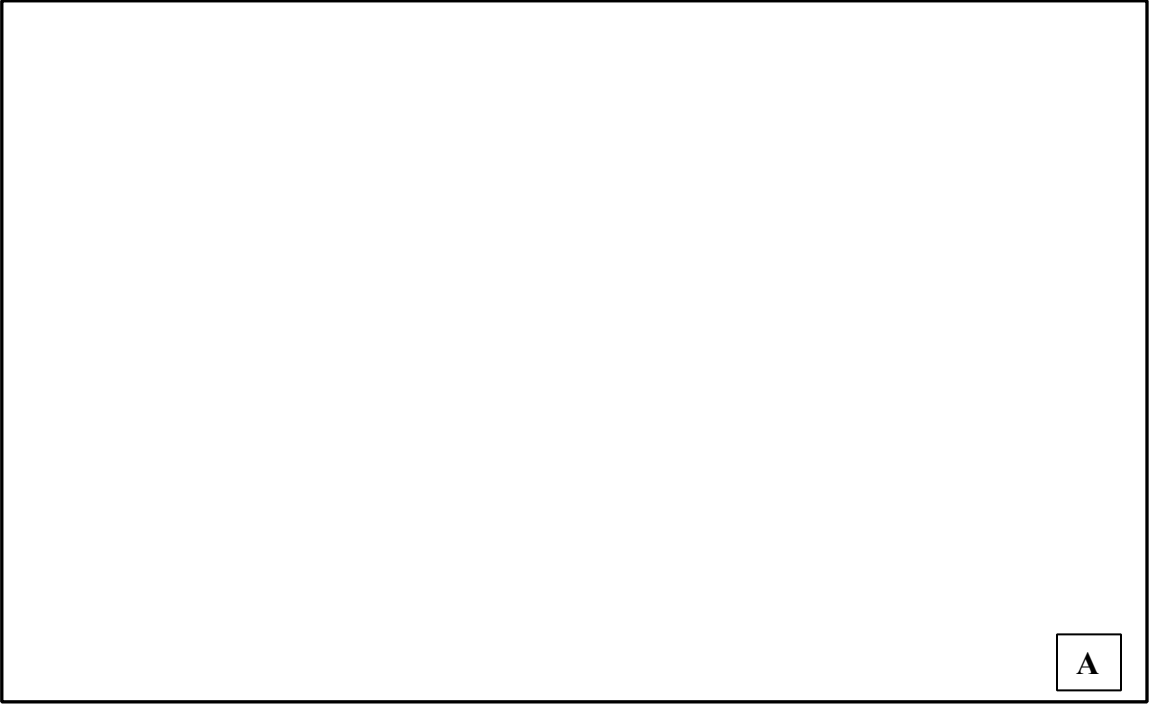


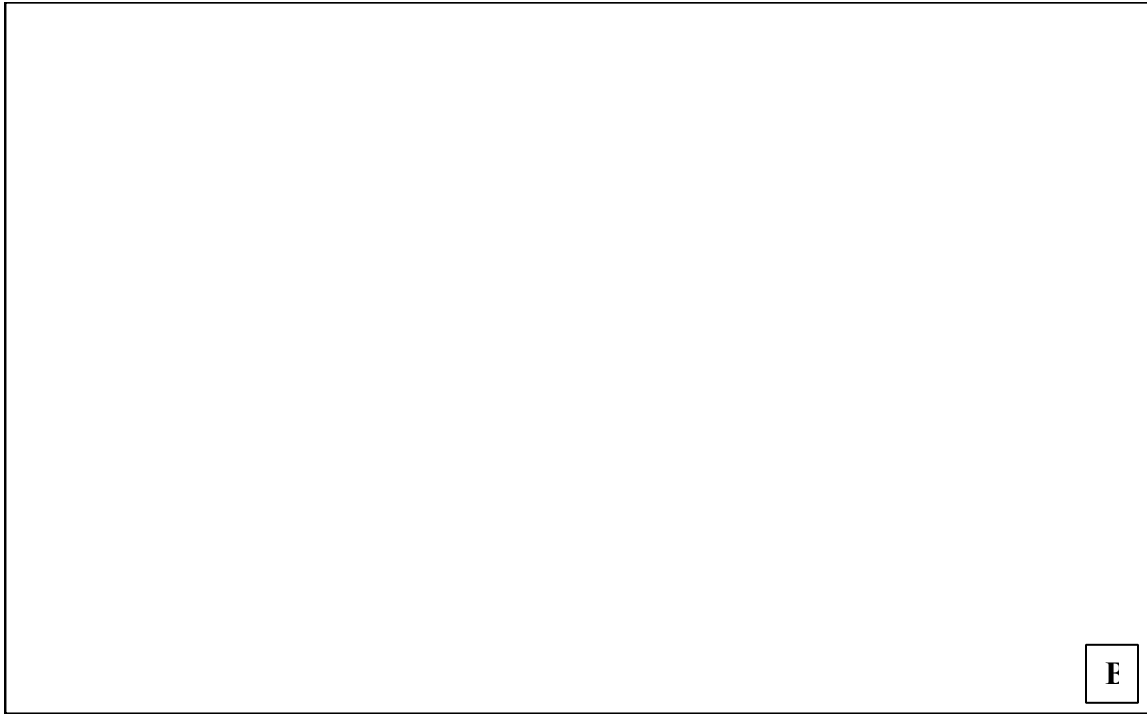
**Figure 3:** Effect of bacterial wilt disease inoculation on plant height (cm) among rootstocks and self rooted Anna F1 control at 18 WAT at **(A)** Chesingoro, and at **(B)** Kapkatet site. Graphs with same letters indicate no graphs indicate no significant difference, according to Tukey's HSD range test ( $p \geq 0.05$ ).

## **4.2 Effect of grafting on bacterial wilt disease**

### **4.2.1 Disease incidence on grafted tomato**

Bacterial wilt disease symptoms were observed among inoculated grafts from 14 WAT, as was the case for inoculated rootstocks. While PWP reached 100% in un-grafted 'Anna F1' at 18 WAT at Chesingoro (Figure 4A), there was no wilting on tomato plants grafted on wild *S. aethiopicum* and DB3 rootstocks in both study sites (Figure 4; Appendix I-2). Conversely, plants grafted on goat apple and wild tomato had up to 44% and 11% PWP, respectively (Figure 4; Appendix I-2). Grafting on all four rootstocks decreased the wilting incidence effects on Anna F1, compared to un-grafted control plants, across both study sites, at 18 WAT. The same effect was significant at Kapkatet, at 14 and 16 WAT (Figure 4; Appendix I-2). However, goat apple grafted plants were observed to be significantly less ( $p > 0.05$ ) wilt tolerant compared to grafts of wild *S. aethiopicum* and DB3 rootstocks at 18 WAT, at Chesingoro site (Figure 4; Appendix I-2).





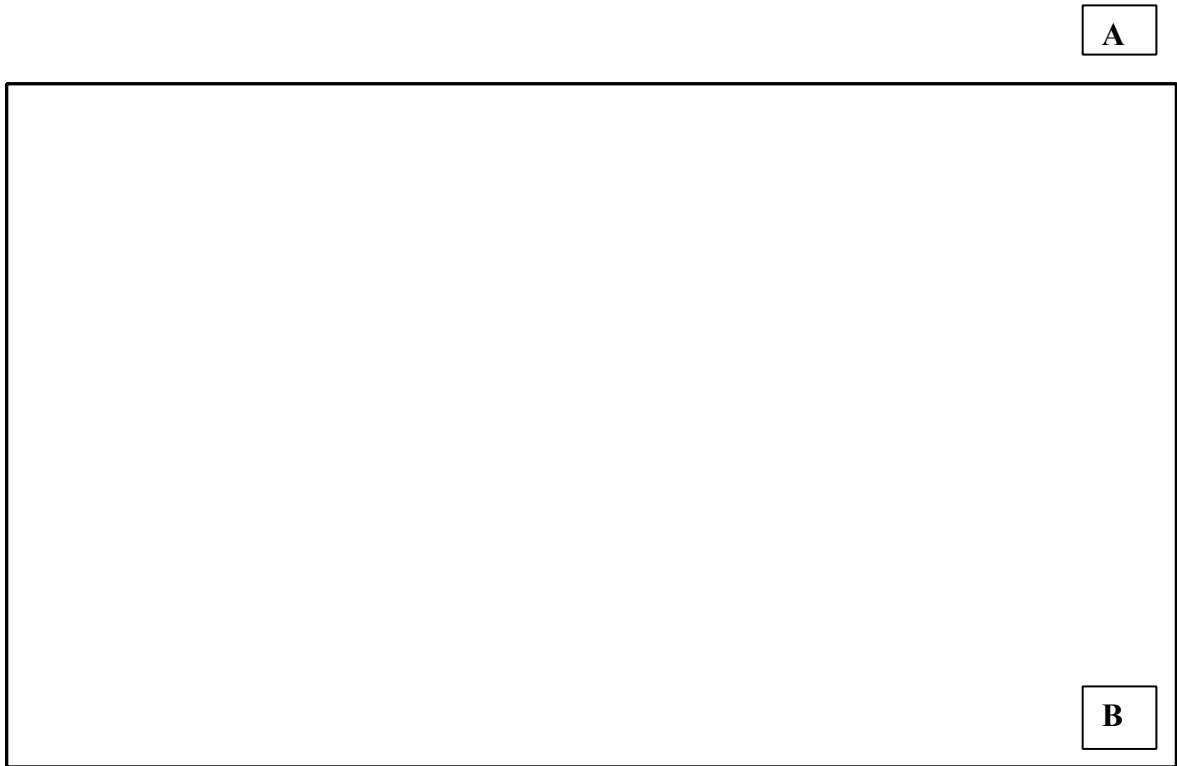
**Figure 4:** Disease incidence, observed as PWP, among inoculated grafts in **(A)** Chesingoro site, and **(B)** Kapkatet site.

#### **4.2.2 Effect of grafting on bacterial wilt severity**

Grafting on each of the four rootstocks significantly ( $p < 0.05$ ) reduced bacterial wilt disease severity, in comparison to un-grafted AnnaF1 control plants in both sites (Figure 5; Appendix I-2). Wild *S. aethiopicum* and DB3 grafts had no disease symptoms in both sites. At Chesingoro site, which recorded higher disease levels, wilt severity in goat apple grafts reached 40% PDI at 18 WAT, compared to 10% PDI for those grafted on wild tomato rootstocks and 82.7% PDI for control plants.



Goat apple PDI value (40% PDI) at 18 WAT was comparable to that of wild tomato, but significantly ( $p<0.05$ ) higher than for the other grafts, at Chesingoro site (Figure 5; Appendix I-2). At Kapkatet wilt severity on un-grafted Anna F1 plants reached 54.4%, which was significantly ( $p<0.05$ ) higher compared to 7.8% in both wild tomato and goat apple grafts, at 18 WAT. While grafts were not significantly different with respect to PDI at Kapkatet site, from 14-18 WAT, the PDI values indicated significantly higher disease severity on un-grafted Anna F1 plants, than on all the grafts, at these growth stages (Figure 5; Appendix I-2).



**Figure 5:** Disease severity observed as PDI, among inoculated grafts, during 14-18WAT, at Chesingoro site (A), and at Kapkatet site (B).

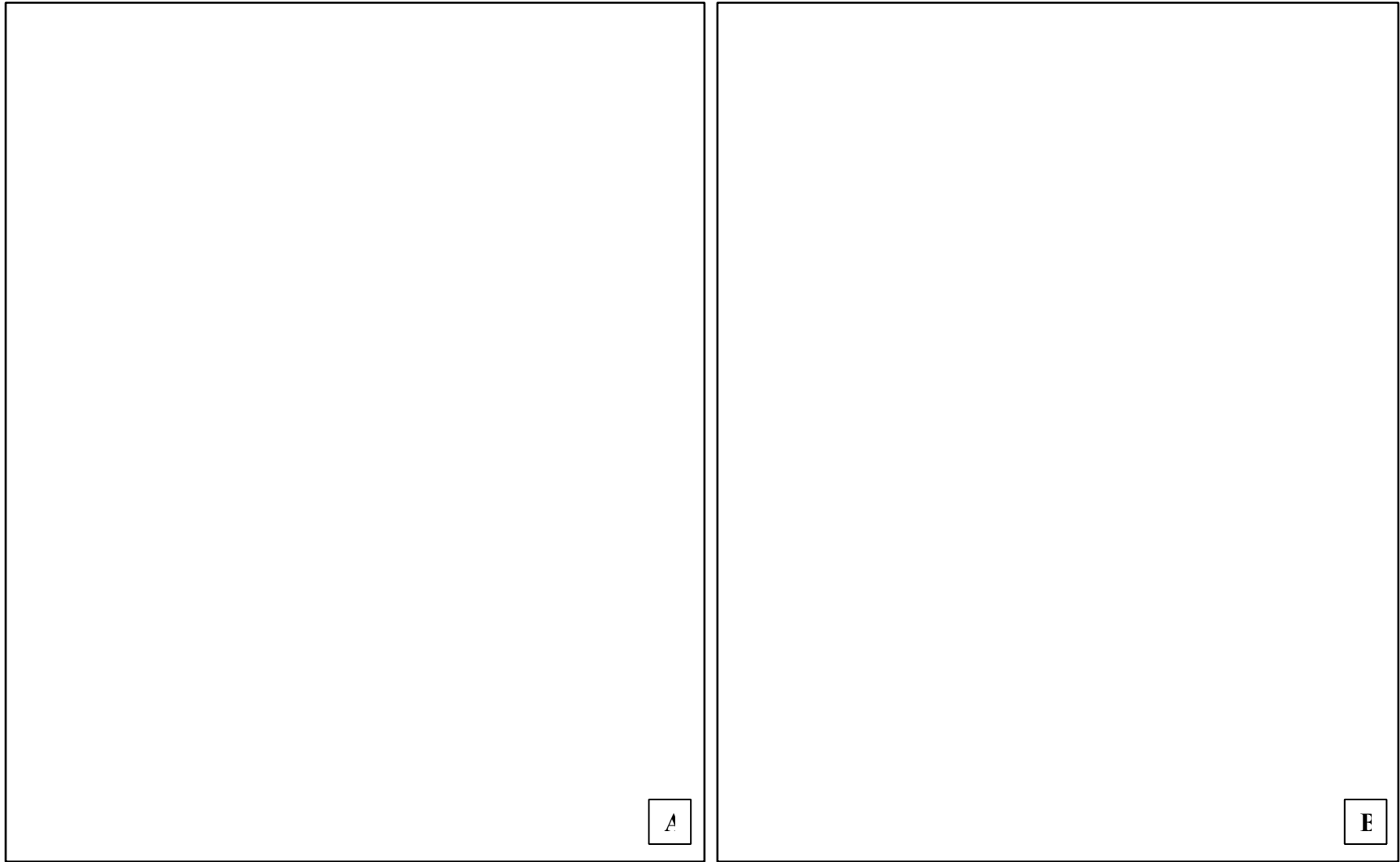
### **4.3 Effect of grafting on growth, yield and quality of tomato**

#### **4.3.1 Effect of grafting on plant height of tomato**

At Chesingoro site, the main vegetative growth stages brought all grafts and un-grafted Anna F1 plants to a comparable height by eight WAT, regardless of their initial plant height at transplanting (Figure 6A; Appendix I-4). During growth stages from 14-18 WAT, achieved plant height among inoculated grafts of goat apple and wild tomato, and un-grafted ‘Anna F1’ plants were mainly reduced, while wild *S. aethiopicum* and DB3 grafts

were least affected (Figure 6B), in comparison to non-inoculated sets of each kind (Figure 6A; Appendix I-4), coincidentally with observation of bacterial wilt disease symptoms.

At 18 WAT, grafts of wild *S. aethiopicum* produced the tallest plants, at Chesingoro site, with minimal decrease in height (10.5cm), compared to non-inoculated plants (Figure 6A; Appendix I-4) and inoculated plants (Figure 6B; Appendix I-4). This was against height difference of 14.9 cm between non-inoculated un-grafted Anna F1 control plants (Figure 6A, Appendix I-4), and inoculated Anna F1 plants (Figure 6B; Appendix I-4). DB3 grafts also outgrew un-grafted Anna F1 control plants, and grafts of goat apple and wild tomato plants, at 18 WAT. At the same growth stage, inoculated wild tomato plants were the shortest amongst all grafts(Figure 6B; Appendix I-4), with a height decrease of 20.6 cm height difference in comparison to non-inoculated set of the same plants (Figure 6A; Appendix I-4). The largest height reduction (by 27.7 cm) was observed between non-inoculated (Figure 6A; Appendix I-4) and inoculated goat apple grafts (Figure6B; AppendixI-4).



**Figure 6:** Effect of grafting to different rootstocks on plant height of tomato, from transplanting stage (0 WAT) to 18 WAT: **A:** in absence of bacterial wilt disease inoculation; and **B:** under disease inoculation, at Chesingoro site

At Kapkatet site, plant heights followed the same trend as at Chesingoro site, except that goat apple grafts achieved higher plant height compared to DB3 grafts during all growth stages, both in absence of bacterial wilt disease inoculation (Figure 7A; Appendix I-4), and under disease inoculation conditions (Figure 7B; Appendix I-4). All height differences observed between inoculated plants and non-inoculated sets of each kind of plants were, however, not statistically significant ( $p \geq 0.05$ ) at all the growth stages, at both sites (Appendix I-4).

**Figure 7:** Effect of grafting to different rootstocks on plant height of tomato, from transplanting stage (0 WAT) to 18 WAT: **(A)** in absence of bacterial wilt disease inoculation; and **(B):** under disease inoculation, at Kapkatet site

A

B

#### 4.3.2 Effect of grafting on plant growth rates of tomato

At Chesingoro site, plant growth rates at the beginning of the vegetative stage, at 2 WAT after transplanting, were significantly higher for Un-grafted Anna F1 and wild tomato grafts, in comparison to initial growth rates among other grafts (Figure 8; Appendix I-5). However, growth rates among all grafts were not significantly different compared to un-grafted Anna F1 plants during the main vegetative growth stages (4 to 8 WAT) (Figure 8; Appendix I-5). At 4 WAT, the growth rates among non-inoculated grafts narrowly ranged from 25.1 cm/ fortnight (DB3 grafts) to 30.4 cm/fortnight (wild tomato grafts), compared to that of Anna F1 control plants (27.8 cm/fortnight), while at 8 WAT, this range was 21.4 cm/fortnight (wild *S. aethiopicum* ) to 28.8 cm/ fortnight (un-grafted Anna F1)(Figure 8; Appendix I-5).

During the stages from 14 to 18 WAT, when bacterial wilt disease symptoms were also observed, the growth rates of inoculated un-grafted Anna F1, and inoculated grafts of goat apple grafts and wild tomato grafts were lower, compared to those of inoculated grafts of DB3 and wild *S. aethiopicum*, though not significantly ( $p \geq 0.05$ ), at Chesingoro site (Appendix I-5). At 18 WAT, the growth rate of inoculated un-grafted Anna F1 was the lowest at 1.2 cm/fortnight, followed by inoculated wild tomato grafts (1.8 cm/fortnight), in contrast to higher growth rates among wild *S. aethiopicum* (7.7 cm/fortnight) and DB3 (4.7 cm/fortnight). At the same growth stage, non-inoculated grafts of wild *S. aethiopicum* and DB3 also attained higher (but not significantly) growth rates of 10.9 cm/fortnight and 10.8 cm/fortnight, respectively, compared to those of non-inoculated un-grafted Anna F1 (6.9 cm/fortnight), goat apple grafts (5.6 cm/fortnight) and wild tomato grafts (3.8 cm/fortnight) (Figure 8; Appendix I-5).

**Figure 8:** Plant growth rates observed at Chesingoro site for A: C

**A**

**B**



At Kapkatet site, Anna F1 control plants also achieved higher growth rates, initially, compared to all grafts, attaining 32.5 cm/ fortnight and 32.5 cm/fortnight among non-inoculated and inoculated sets respectively, at 4 WAT (Figure 9; Appendix I-5). However, at 8 WAT, all grafts achieved higher growth rates than the un-grafted tomato plants (Figure 9; Appendix I-5). DB3 grafts achieved the highest growth rate (34 cm/fortnight) which was significantly higher than for un-grafted Anna F1 tomato (18.6 cm/ fortnight), comparing non-inoculated plants (Figure 9; Appendix I-5). A similar trend in growth rates were also observed among inoculated plants at 8 WAT.

During growth stages from 14 to 18 WAT, representing the stages at which visible disease symptoms were observed, all inoculated grafts attained higher growth rates in comparison to inoculated un-grafted tomato control plants (Figure 9). At 18 WAT, the growth rate of inoculated un-grafted Anna F1 was 6.1 cm per fortnight, compared to grafts of wild *S. aethiopicum* (19.5 cm/fortnight), DB3 (15.5 cm/fortnight), goat apple (14.7 cm/fortnight) and wild tomato (7.7 cm/fortnight) (Figure 9; Appendix I-5). However, no significant difference ( $p \geq 0.5$ ) was observed, comparing growth rates for inoculated and non-inoculated sets within the same kinds of plants; and also comparing growth rates across the different kinds of both inoculated and non-inoculated plants (Figure 9; Appendix I-5)

**Figure 9:** Plant growth rates observed at Kapkatet site for A: Gr

**A**

**B**

### 4.3.3 Effect of grafting on total yields

While bacterial wilt disease inoculation caused a significant decrease in the yield of the un-grafted ‘Anna F1’ control plants, in both sites, all graft combinations inoculated with the disease recorded no significant ( $p \geq 0.05$ ) yield decrease, in comparison to corresponding non-inoculated sets of plants (Table 1).

**Table 1: Total fruit yield of tomato grafts and control plants**

Site	Grafting combination	Yield of picked fruits (g/plant)		
		Non-inoculated plants	Inoculated plants	
Chesingoro	Wild <i>S. aethiopicum</i> x Anna F1			
			780 ab <sup>1</sup>	723 ab
	DB3 x ‘Anna F1’	1023 a	772 ab	
	Wild tomato x ‘Anna F1’	858 ab	705 ab	
	Goat apple x ‘Anna F1’	819 ab	484 b	
	Un-grafted ‘Anna F1’ (control)	976 a	510 b	
	Tukey’s range test MSD = <b>463.4 g</b>			
Kapkatet	Wild <i>S. aethiopicum</i> x Anna F1			
			503 ab	469 abc
	DB3 x ‘Anna F1’	408 abcd	380 abcd	
	Wild tomato x ‘Anna F1’	357 bcd	308 d	
	Goat apple x ‘Anna F1’	347 cd	326 cd	
	Un-grafted ‘Anna F1’ (control)	525 a	266 d	
	Tukey’s range test MSD = <b>150.4 g</b>			

MSD= Minimum significant difference

<sup>1</sup>Means followed by the same letter (s) are not significantly different, according to Tukey’s HSD range test ( $p \geq 0.05$ ).

Under disease conditions, only grafting on wild *S. aethiopicum* significantly increased the yield of ‘Anna F1’, only in Kapkatet site (Table 1). Grafting on DB3 and wild tomato rootstocks increased the yield of ‘Anna F1’, under disease conditions, across both sites, but not significantly. Comparing bacterial wilt disease inoculated plants, the highest yields obtained in Chesingoro site were from DB3 grafts followed by wild *S. aethiopicum* grafts. These corresponded to yield increase of 51.2% and 41.7%, in comparison to yield of inoculated ‘Anna F1’ control plants, respectively (Table 6). The highest yields with respect to inoculated plants at Kapkatet site were obtained from wild *S. aethiopicum* grafts, followed by DB3 grafts, corresponding to yield increase of 76.3% and 42.7%, respectively, in comparison to yield of 266g/ plant for inoculated un-grafted ‘Anna F1’ (Table 1). Yields of inoculated wild tomato grafted plants were 38.1% and 15.8% higher compared to control at Chesingoro and Kapkatet sites, respectively (Table 1). Under disease conditions, goat apple grafted plants had 22.6% higher yield, compared to un-grafted ‘Anna F1’ at Kapkatet site, but recorded a 5.3% lower yield, compared to un-grafted Anna F1 at Chesingoro site (Table 1).

In absence of disease conditions, the highest yield of 1023g/plant was obtained from grafts of DB3 rootstock at Chesingoro site, which was higher but not significantly different from yields of un-grafted ‘Anna F1’, and other graft combinations (Table 1). At Kapkatet site, the highest yield of 525g/plant was obtained from ‘Anna F1’ plants, which was not significantly different from yield of wild *S. aethiopicum* grafted plants, and DB3 grafted plants, but significantly higher than yields from wild tomato and goat apple grafted plants (Table 1). At Chesingoro site, the highest yield decrease in comparison to un-grafted ‘Anna F1’ control plants was recorded by wild *S. aethiopicum* grafts (22.1%), followed by goat apple grafts (16.1%), while at Kapkatet site, decrease in yield in comparison to un-grafted control reached 33.9% and 32% in goat apple and wild tomato grafts, respectively (Table 1). These yields were significantly ( $p < 0.05$ ) lower compared to control (Table 1).

#### **4.3.4 Effect of grafting on yield related attributes (Fruit set; Time to onset of harvesting; number of fruits picked per plant; and average fruit weight)**

##### **4.3.4.1 Effect of grafting on Fruit set in tomato**

In absence of disease conditions, grafting did not significantly ( $p \geq 0.05$ ) affect the number of fruits set, number of clusters per plant, and the average fruit set per cluster across all the study sites. However, under disease conditions all the grafts, except for DB3 produced significantly ( $p \geq 0.05$ ) higher number of clusters per plant, compared to un-grafted 'Anna F1' control, at Kapkatet site, while the same were all comparable in Chesingoro site (Table 2). At Chesingoro site, DB3 grafts set the highest total number of fruits (19.8) borne in seven clusters, hence an average of 3 fruits per cluster, compared to 15 fruits borne in five clusters with average of three fruits per cluster among the control plants, comparing non-inoculated set of plants (Table 2).

At Kapkatet site, the highest fruit set was observed in wild *S. aethiopicum* grafts which set 26 fruits per plant borne in seven clusters, averaging four fruits per cluster, under no disease inoculation, compared to 18 fruits per plant in six clusters with three fruits per cluster among 'Anna F1' control plants (Table 2). This rootstock also had the most clusters, and a significantly greater number of fruits set, compared to control, under disease inoculation, in the same site (Table 2).

##### **4.3.4.2 Effect of grafting on time to onset of harvesting of tomato fruits**

Regardless of disease conditions, grafting had no effect on the duration from transplanting to onset of harvesting, across both sites, in comparison to un-grafted control. Pooling all the different types of grafts and un-grafted 'Anna F1', fruits reached initial harvest at

colour break stage of maturity at 53-66 DAT in Chesingoro site, while in Kapkatet site, this ranged from 61-69 DAT (Table 2).

**Table 2: Mean observations of yield related attributes for grafted tomato plants and control at, 130 DAT**

Site	Grafting <sup>a</sup> rootstock	Bacterial wilt disease inoculation	No. of fruits set/ plant	No. of clusters/ plant	No. of fruits set/ cluster	Days to 1 <sup>st</sup> harvest	No. of fruits picked/ plant	Fruit weight (g)
Chesingoro	Wild <i>S. aethiopicum</i>	Not inoculated	16.0ab <sup>1</sup>	6.3ab	2.5a	54c	15.0a	51.5ab
		Inoculated	13.9ab	5.7ab	2.4a	56bc	12.7a	57.8ab
	DB3	Not inoculated	19.8a	6.8a	2.9a	53 c	17.7a	58.5ab
		Inoculated	17.5ab	5.9ab	3.0a	54c	15.6a	49.2ab
	Wild tomato	Not inoculated	15.3ab	5.1ab	3.0a	63ab	14.7a	58.9ab
		Inoculated	14.5ab	5.0ab	2.9a	56bc	14.3a	49.6ab
	Goat apple	Not inoculated	17.7ab	5.3ab	3.4a	66a	16.9a	48.3ab
		Inoculated	14.1ab	4.2ab	3.3a	57bc	13.7a	35.5b
	Un-grafted ‘Anna F1’ (control)	Not inoculated	15.1ab	5.0ab	3.1a	58abc	13.9a	69.8a
		Inoculated	11.5ab	4.4ab	2.7a	58abc	11.5a	45.3b
Minimum significant difference <sup>3</sup>			9.0	7.2	8.0	2.1	1.7	24.0
Kapkatet	Wild <i>S. aethiopicum</i>	Not inoculated	25.8a	6.9a	3.8a	61a <sup>1</sup>	21.9a	23.6b
		Inoculated	22.6ab	6.7a	3.4ab	63a	17.4ab	26.9ab
	DB3	Not inoculated	13.3cd	5.1ab	2.6abc	67a	11.8bcd	34.6a
		Inoculated	14.6bcd	5.3ab	2.7abc	65a	11.6bcd	32.8ab
	Wild tomato	Not inoculated	13.6cd	6.1a	2.2bc	63a	11.2cd	32.2ab
		Inoculated	11.0cd	5.8a	1.9c	69a	8.6bcd	35.9a
	Goat apple	Not inoculated	17.9acb	6.0a	3.0abc	64a	14.4bcd	24.0b
		Inoculated	14.7bcd	6.3a	2.3bc	62a	11.8bcd	27.6ab
	Un-grafted ‘Anna F1’ (control)	Not inoculated	18.4abc	6.0a	3.1abc	68a	14.6bc	36.1a
		Inoculated	7.8cd	3.6b	2.2bc	69a	7.8d	34.6a
Minimum significant difference			10.5	6.7	8.6	2.1	1.4	9.3

<sup>a</sup>Scions of 'AnnaF1' tomato variety were grafted to each rootstock <sup>1</sup>Values in each column followed by the same letter (s) are not significantly different, according to Tukey's HSD range test ( $p \geq 0.05$ ). <sup>3</sup>Tukey's range test minimum significant difference



#### **4.3.4.3 Effect of grafting on number of fruits picked per plant in tomato**

Irrespective of disease condition, grafting on any of the rootstock did not significantly affect the number of fruits produced in comparison to un-grafted plants, at Chesingoro site. The same case applied at Kapkatet site, except that wild *S. aethiopicum* grafted plants recorded significantly ( $p>0.05$ ) higher fruit numbers over control (Table 2). Wild *S. aethiopicum* recorded the fruit number of fruits averaging 21.9 and 17.4 fruits per plant, among non-inoculated and inoculated plants respectively, compared to 14.6 and 7.8 fruits per plant for non-inoculated and inoculated control plants respectively, at Kapkatet site (Table 2).

At Chesingoro site, DB3 produced the largest number of fruits averaging 17.7 fruits/plant and 15.6 fruits per plant, among non-inoculated and inoculated plants respectively, compared to 13.9 fruits/plant and 11.5 fruits/plant among ‘Anna F1’ control plants (Table 2). However, the difference in fruit numbers between DB3 and the control plants was not significant (Table 2).

#### **4.3.4.4 Effect of grafting on average fruit weight in tomato**

Under disease conditions, grafting on any of the rootstocks did not significantly ( $p>0.05$ ) influence fruit weights compared to un-grafted control plants, across both sites. Wild *S. aethiopicum* produced the heaviest fruits (57.8g) among inoculated grafts at Chesingoro site, but was inconsistent, also producing the lightest fruits (26.9g) in Kapkatet site (Table 2). Fruits of inoculated un-grafted ‘Anna F1’ plants averaged 45.3g and 34.6g at Chesingoro site and Kapkatet site, respectively (Table 2).

In absence of disease, non-inoculated ‘Anna F1’ control plants had heavier fruits than grafts, averaging 69.8g and 36.1 g at Chesingoro and Kapkatet sites, respectively (Table 2). Among non-inoculated grafts, wild tomato grafts produced the heaviest fruits (58.9g) in Chesingoro site (Table 2); while fruits of DB3 grafts were the heaviest, at Kapkatet site (34.6g) (Table 2). Under inoculation, fruit weights for all grafts were comparable to control, at Chesingoro site. However, wild *S. aethiopicum* grafts and goat apple grafts had

significantly lower fruit weight compared to control at Kapkatet site (Table 2). Therefore, wild *S. aethiopicum* and goat apple grafts showed a characteristic of producing large numbers of fruits per plant, but having the lightest individual fruit weights, at Kapkatet site (Table 2).

#### **4.3.5 Effect of grafting on quality of tomato fruits**

##### **4.3.5.1 Effect of grafting on Fruit size in tomato**

Comparing inoculated plants, grafting on all rootstocks except goat apple produced fruits whose diameter were not different from un-grafted ‘Anna F1’ control plants, across both study sites (Table 3). Goat apple grafted fruits were significantly reduced in size compared to control, at both sites (Table 3). In addition, among non-inoculated plants, grafting on each of the rootstocks significantly ( $p < 0.05$ ) decreased fruit diameter at Chesingoro site, but this was not apparent at Kapkatet site (Table 3).

The largest fruits were produced by the un-grafted ‘Anna F1’ plants, regardless of disease conditions, with fruit diameters averaging 4.60 cm and 4.22 cm among inoculated and non-inoculated plants at Chesingoro site, respectively, while this was 3.71cm and 3.83cm respectively, at Kapkatet site (Table 3). Non-inoculated grafts recorded fruit diameters ranging from 3.99cm - 4.19 cm and 3.39cm - 3.76cm at Chesingoro and Kapkatet sites, respectively (Table 3), while this range was 3.73 cm – 4.18 cm and 3.39 – 3.81 among inoculated grafts at Chesingoro and Kapkatet sites, respectively (Table 3). DB3 and wild tomato grafted fruits were always larger than goat apple and wild *S. aethiopicum* grafted fruits.

##### **4.3.5.2 Effect of grafting on pH values of tomato fruits**

Regardless of disease conditions, there was no significant difference between the pH of fruit pulps of each graft, compared to un-grafted control plants, across both sites (Table 3). The pH of grafts was comparable across both sites, ranging from 4.44 to 4.72 and from 4.39

to 4.75, at Chesingoro and Kapkatet sites, respectively (Table 3). Additionally, the pH of the ‘Anna F1’ control plants obtained from non- inoculated and inoculated plants were 4.43 and 4.54 respectively at Chesingoro site; and 4.63 and 4.58, respectively, at Kapkatet site (Table 3).

**Table 3: Quality characteristics of fruits picked at colour break ripening stage, 90 DAT**

Site	Bacterial wilt disease Inoculation	Grafting rootstock		Fruit diameter (cm)	PH	BRIX
Chesingoro	Not inoculated	Wild	<i>S.</i>	4.01bc <sup>1</sup>		
		<i>aethiopicum</i>			4.44a	5.46d
		DB3		4.19b	4.65a	5.96bcd
		Wild tomato		4.19b	4.66a	5.73cd
		Goat apple		3.99bc	4.50a	6.31abc
	Inoculated	Un-grafted ‘Anna F1’		4.60a	4.43a	6.02bcd
		Wild	<i>S.</i>	3.92bc		
		<i>aethiopicum</i>			4.45a	6.14bc
		DB3		4.05bc	4.49a	5.70cd
		Wild tomato		4.18b	4.65a	6.49ab
		Goat apple		3.73c	4.72a	5.91bcd
		Un-grafted ‘Anna F1’		4.22ab	4.54a	6.95a
		Minimum significant difference (Tukey’s range test)			0.39	0.29
Kapkatet	Not inoculated	Wild	<i>S.</i>	3.63abc <sup>1</sup>		
		<i>aethiopicum</i>			4.45ab	6.42ab
		DB3		3.76ac	4.46ab	6.49ab
		Wild tomato		3.68abc	4.75a	7.11a
		Goat apple		3.43bc	4.68ab	7.20a
	Inoculated	Un-grafted ‘Anna F1’		3.71abc	4.63ab	6.59ab
		Wild	<i>S.</i>	3.48abc		
		<i>aethiopicum</i>			4.58ab	5.41b
		DB3		3.72abc	4.39b	7.67a
		Wild tomato		3.81ab	4.60ab	7.55a
		Goat apple		3.39c	4.45ab	6.48ab
		Un-grafted ‘Anna F1’		3.83a	4.58ab	6.61ab

Minimum significant difference (Tukey's range test)	0.39	0.33	1.52
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<sup>1</sup>Values in each column followed by the same letter (s) are not significantly different, according to Tukey's HSD range test ( $p \geq 0.05$ ).

#### 4.3.5.3 Effect of grafting on Brix value of tomato fruits

At Chesingoro site, fruit Brix values under disease conditions for grafts of wild *S. aethiopicum* (6.14 B<sup>o</sup>), DB3 (5.70 B<sup>o</sup>), and goat apple (5.91 B<sup>o</sup>) were significantly ( $p < 0.05$ ) reduced in comparison to un-grafted 'Anna F1' control plants (6.95 B<sup>o</sup>), but wild tomato grafted fruits recorded a higher brix value of 6.49 B<sup>o</sup>, which was not significantly different from inoculated control fruits (Table 3).

At Kapkatet, all inoculated grafts indicated no significant difference with respect to fruit Brix, in comparison to control. DB3 grafted fruits recorded the highest value of 7.67 B<sup>o</sup>, followed by wild tomato, with 7.55 B<sup>o</sup>, against 6.61 B<sup>o</sup> for control (Table 3).

In absence of disease conditions, all grafts did not significantly differ with respect to Brix, in comparison to non-inoculated control plants (Table 3). Goat apple grafted fruits had the highest Brix values of 6.31 B<sup>o</sup> and 7.20 B<sup>o</sup>, which were comparable to 6.02 B<sup>o</sup> and 6.59 B<sup>o</sup> among control plants, at Chesingoro and Kapkatet sites, respectively (Table 3).

## CHAPTER 5

### DISCUSSION

#### 5.1 Bacterial wilt disease tolerance in rootstocks

In this study, the local wild *S. aethiopicum* accession and DB3 completely tolerated bacterial wilt disease, since inoculated plants showed no wilt symptoms, and is consistent with reported use of these African eggplant genotypes as highly tolerant and against bacterial wilt disease worldwide (Ibrahim *et al.*, 2001; Besri, 2002; Schippers, 2004; MBOTC, 2006). The findings indicated that the *S. aethiopicum* genotypes tested are resistant to the local strains of *R. solanacearum* endemic in the study area. Though specific race and biovar classification of *R. solanacearum* pathogen involved was not established in this study, previous studies identified race 3, biovar 2A, in similar high altitude areas, associated with potato cropping systems in Western Kenya (Rotich, 2010).

A moderate level of tolerance to bacterial wilt disease was observed among the local wild tomato genotype (*Solanum lycopersicum*) tested, with only up to 22% incidence level recorded, which was lower in comparison to 83% incidence among control tomato plants. Similar small fruited wild tomato genotypes have previously been found to have tolerance to bacterial wilt, particularly those belonging to *Lycopersicum pimpinellifolium* and *L. esculentum* var. *Cerasiforme* species (Wang *et al.*, 1998; Oda, 1999; USDA, 2006). However, just a few of these genotypes, most notably Hawaii 7996-7998 (*Lycopersicum Pimpinellifolium*), and Caraibo (France) line CRA 66 (*L. esculentum* var. *Cerasiforme*) have so far been found to be highly resistant, whereas FL7514 and L285 (*L. esculentum* var. *Cerasiforme*) have only moderate tolerance (Grimault *et al.*, 1994; Wang *et al.*, 1998; Rivard and Louws, 2006; USDA, 2006). These resistant small-fruited tomato lines have been utilized as genetic resource germplasm for bacterial wilt tomato cultivar breeding programs at AVRDC (Opena *et al.*, 1990; Scott *et al.*, 2005).

Goat apple (*S. aculeastrum*) was found to be partially bacterial wilt tolerant, with lower wilting incidence of 44% observed, against 100% wilt incidence in the control tomato cultivar. Evaluation of this wild solanum species as a potential rootstock against bacterial wilt disease has not been previously reported. Previous research have shown varying levels of tolerance among other wild solanum genotypes, including those belonging to *S. indicum*, *S. torvum*, *S. incunum*, *S. integrifolium*, *S. macrocarpon*, *S. sanitwongswai*, *S. sisybriifolium* and *S. melongena* species (Ibrahim *et al.*, 2001; Magambo *et al.*, 2002; Schippers, 2004). Goat apple genotype evaluated showed a lower tolerance level in comparison with wild *S. melongena* 'EG203' genotype, in which lower incidence levels of 7% was observed under high disease pressure in one study in Philippines (Santiago *et al.*, 2002). 'EG 203' is considered a highly resistant rootstock recommended for the grafting of tomato, against bacterial wilt disease in Asia (Black *et al.*, 2003). In Uganda, another study also reported high tolerance with nil wilt bacterial wilt disease incidence among inoculated plants of *S. indicum* subsp *distichum* and *S. macrocarpon*, where a susceptible tomato cultivar (*Lycopersicon esculentum*) had 100% wilt incidence (Magambo *et al.*, 2002). However this study found 75% wilt incidence on *S. incunum* plants, hence ranking lower in comparison to tolerance of goat apple observed in the current study.

Plant height of inoculated rootstocks was reduced in all cases, in comparison to non-inoculated plants of each type, at 18 WAT. Similar findings were reported by Magambo *et al.* (2002), who observed reductions in plant height and number of leaves among inoculated plants of *S. macrocarpon*, *S. indicum* subsp *dischitum*, *S. incunum* and *S. camphyllocathum* in comparison to non-inoculated control plants. Magambo *et al.* (2002), observed height reduction among inoculated *S. macrocarpon*, *S. indicum* subsp *dischitum* and *S. camphyllocathum* despite the absence of visible wilt symptoms. A similar observation was apparent in the current study, since inoculated wild *S. aethiopicum* and DB3 plants were symptomless, but showed height reduction. The reduced plant height effect showed that symptomless plants were highly tolerant, but not immune to bacterial wilt disease (Magambo *et al.*, 2002). It was previously reported that resistant host plants were not immune to bacterial wilt infection, but rather, multiplication of the pathogen is reduced in the vascular system of tolerant genotypes (Grimault *et al.*, 1994; Nakaho *et al.*,

2000). The precise physiological and molecular mechanisms responsible for resistance against bacterial wilt disease in host plants have not been clearly established. So far, research has suggested that tylose formation in resistant host plants physically limit entry of the pathogen from the soil environment into the secondary xylem tissues of the roots, and its movement into the collar and mid-stem of the host plant (Grimault *et al.*, 1994; Nakaho *et al.*, 2000).

## **5.2 Effect of grafting on bacterial wilt disease of tomato**

The popular greenhouse grown tomato cultivar, ‘Anna F1’, was found to be susceptible to bacterial wilt, with up to 100% incidences observed at Chesingoro site. Research has shown that tomato resistance is only confined to small fruited wild genotypes which are unsuitable commercially, such as the Hawaii lines (Opena *et al.* 1990; Wang *et al.*, 1998). Because oligogenic resistance is involved, attempts to integrate the responsible combination of several dominant and recessive resistance genes from such genotypes into the popular large fruited cultivars have so far, been unsuccessful (Scott *et al.*, 2005). Therefore, only a few commercial cultivars having much less resistance compared the resistant non commercial genotypes have been developed globally, such as ‘Neptune’, derived from Hawaii 7997 (Scott *et al.*, 1995).

In this study, grafts of African eggplant genotypes tested (wild *S. aethiopicum* and DB3), totally controlled bacterial wilt disease in grafts with the hybrid tomato cultivar, ‘Anna F1’. These genotypes have already been recommended by various authorities for inter-specific grafting to susceptible tomato genotypes, against bacterial wilt and a wide range of other soil borne biotic stressors, including bacterial wilt, Fusarium wilt, Verticillium wilt and root-knot nematodes, as well as against abiotic stressors such as floods, typhoons, and soil temperature extremes; for which they have shown multiple tolerance (Williams *et al.*, 1991; Besri, 2002; Black *et al.*, 2003; AVRDC, 2005; MBTOC, 2006).

Grafts of the local wild tomato were found to be moderately wilt-tolerant, with 11% disease incidence, against up to 100% incidence in the un-grafted ‘Anna F1’ cultivar. This

was comparable to 6.7% incidences observed in the wild tomato genotype, *S. sisymbriifolium* previously reported by Ibrahim *et al.* 2001. However, this level of tolerance was slightly lower compared to no incidences in grafts of CRA 66 and Hawaii 7996 wild tomato genotypes, against 79% incidence levels in ungrafted cultivars, reported by Rivard and Louws (2006). Lopes *et al.* (2006) similarly reported nil incidences on grafts of Hawaii 7996 with 'Santa clara' tomato cultivar, where 80% incidence was observed in the un-grafted cultivar. In separate studies, Tikoo *et al.* (1979) and Grimault and Prior (1994) similarly reported nil bacterial wilt incidence in tomato grafted on CRA 66 rootstocks, against 100% wilt incidences on the un-grafted control cultivars. The fact that a moderate tolerance exist in the local wild tomato genotype investigated was considered an important finding, because, except for a few genotypes such as CRA 66 and Hawaii 7996, reports on bacterial wilt tolerance among tomato genotypes are rare. Other disease tolerant tomato derived interspecific hybrid rootstocks 'Beufort', 'Maxifort', 'Trifort' and 'Herman' (*L. Lycopersicum* x *L. hirsutum*) though widely used commercially against Fusarium and Verticillium wilts, are however not known to be resistant to bacterial wilt disease.

Grafts of the wild local goat apple genotype (*S. aculeastrum*) were partially tolerant, with up to 44.4% wilt incidence observed. Varied levels against bacterial wilt disease have been reported for wild solanum species genotypes as rootstocks in tomato grafts worldwide. In Bangladesh, Ibrahim *et al.* (2001) observed almost no incidences in 'BARI tomato-3' grafted on *S. sinatwongsei*, against 26% bacterial wilt infestation in the un-grafted cultivar, and 6.7% incidence in *S. torvum*. Although in most cases supporting data is unavailable in reports of other authors, Besri (2002) reported that *S. aethiopicum* and *S. torvum* rootstocks are used in the Mediterranean region where bacterial wilt disease is serious, while Schippers (2004), reported that *S. aethiopicum* variety 'Lizuka' is more effective, compared to *S. torvum*. According to Black *et al.* (2003), *S. melongena* var. *esculentum* (eggplant) accessions EG 197 and EG 203 were also effective rootstocks against bacterial wilt in hot humid climates in Asia.

It was evident from the study that the high bacterial wilt tolerance of wild *S. aethiopicum* and DB3 rootstocks impacted comparably high tolerance on their corresponding grafts



with the tomato cultivar 'Anna F1'. Also, wild tomato and goat apple which ranked as partially tolerant rootstocks, impacted the same effects on their grafts. Thus, disease tolerance observed in grafts was directly attributed to the relative tolerance of rootstock used. The mechanisms by which tolerant rootstocks caused tolerance against bacterial wilt in grafts with susceptible tomato cultivars has been studied by various researchers. In one study, reciprocal grafting of resistant tomato scions onto susceptible rootstock resulted in wilt and bacterial colonization by *R.solanacearum*, in the resistant scion, showing that the root system of resistant rootstock physically limited the entry of the pathogen into the scion (Grimault and Prior, 1994). In another study, Lee (1994) similarly observed that suckers and intact adventitious roots from the scion caused scions otherwise grafted on resistant rootstock, to succumb.

The varied tolerance levels to *R. Solanacearum* observed among grafts have been linked to the extent to which colonisation of xylem tissues by the pathogen takes place within the affected plants, depending on the rootstock employed. Prior *et al.* (1996) positively correlated wilt severity and bacterial colonisation index at the collar and mid-stems among genotypes tested. Nakaho *et al.* (2000) further observed that, within resistant genotypes, bacterial colonisation is confined to the primary xylem, and in some cases, such as in the highly tolerant Hawaii 7667, only to the protoxylem, but never occurs in the secondary xylem. In further histopathological studies, Nakaho *et al.* (2004) observed that among highly tolerant rootstock genotypes, *R. solanacearum* moved into scions of grafted plants, but the multiplication of the pathogen in the scion remained below the threshold necessary to show symptoms, hence remain latently infected. In contrast, colonization levels in the scions reached symptomatic levels when grafted on susceptible genotypes (Nakaho *et al.*, 2000).

In this study, the onset of visible disease effects on grafts was delayed beyond the main vegetative (pre-fruiting) stage, since wilt symptoms were observed from 14 WAT. This suggests that environmental conditions were not optimum for disease development. It is suspected that since the test plants were established inside greenhouse, in pots with limited water holding capacity, low soil moisture conditions may have reduced survival of the

pathogen following artificial inoculation. Previous research showed that disease incidences and severity due to *R. solanacearum* required prolonged high soil moisture (Nesmith and Jenkins, 1985). In particular, biovar 2 populations, which were presumed in this study, are known to be sensitive to low moisture conditions (Van Elsas *et al.*, 2005).

While grafting of susceptible tomato cultivars onto resistant tomato or other solanaceous rootstocks has been effective against race 1 strains of *R. solanacearum* and is used commercially in different locations worldwide, the effectiveness of grafting against Race 3 biovar 2 has not been tested (Saddler, 2005; Patrice and Timur, 2008). Previous studies have shown that disease reactions among affected plants vary with the specific race of the pathogen and the environmental conditions. For example, Lopes *et al.* (2006) observed that when H7996 rootstocks were challenged by isolates of biovar 3, 5% wilt incidence was observed against 90% wilt among control plants; while up to 60% incidences against 90% wilting among control plants were observed with biovar 1 isolates. The same study indicated that the resistance of H7996 broke down under a combination of high inoculum levels and high soil temperature and moisture conditions. These findings underscore the need for biovar determination to establish the diversity of the endemic pathogen races, and to screen potential rootstocks and their grafts for tolerance against isolates of the pathogen, under the specific environmental conditions where the pathogen is to be controlled.

### **5.3 Effect of grafting on growth, yield and quality of tomato**

The initial plant heights at transplanting were higher among grafts compared to un-grafted tomato plants. This was accounted for by the added length of rootstock stem bases in grafts. Also, initial plant heights at transplanting varied across grafts. This was mainly attributed to variances in growth rates during the pre-transplanting stage, when the grafts were left to acclimatize and achieve vigorous growth for a period of 2 to 3 weeks before transplanting. Possibly, complete graft healing and acclimatization processes in grafted seedlings varied according to rootstock used. In absence of disease conditions, plant height of both grafts and un-grafted tomato control plants were generally comparable, from 8 to

18 WAT, with exception of wild *S. aethiopicum* grafts, which tended to be taller than the control, at Kapkatet site. Similar results were reported by Khah *et al.* (2006), who found no significant differences in plant height of grafts of tomato 'Big Red' variety on 2 hybrids rootstocks of *Lycopersicon esculentum* ('He-man' and 'Premavera'), under greenhouse conditions, at 130 DAT. At 14 to 18 WAT, inoculated plants were in all cases tended to have reduced height, in comparison to non-inoculated sets of the same kind, due to disease reactions. The height reductions observed showed that inoculated plants were infested with the pathogen in all cases, but grafts with resistant rootstocks tolerated the disease, in agreement with similar previous findings by Magambo *et al.* (2002)

In this study, plant growth rates were considered to be a more important indicator of the growth responses to grafting rootstock and inoculation treatments, both within, as well as across the same kinds of graft combinations, because it was independent of initial plant heights at transplanting. Generally, un-grafted tomato control plants had higher initial (2 to 6 WAT) growth rates compared to grafts, but at later stages (8 to 18 WAT), grafts tended to have higher growth rates than control plants. These observations were consistent with previous findings by other authors who reported that un-grafted plants tended to show higher growth rates initially, due to grafting shock among grafts (Ibrahim *et al.*, 2001; Rivard and Louws, 2006). Under disease conditions, grafts of both wild *S. aethiopicum* and DB3 rootstocks consistently recorded slightly higher growth rates than inoculated un-grafted 'Anna F1' control cultivar, and correspondingly higher yields by 76.3% and 51.2%, over the control, respectively. These findings were consistent with reports by previous authors, who generally reported that eggplant rootstocks were highly effective in controlling bacterial wilt on tomato scions (Besri, 2002; Black *et al.*, 2003; Poffley, 2003; Paladu and Wu, 2005).

Yield among inoculated grafts of wild tomato increased by up to 38%, over control. This result was comparable to those of Ibrahim *et al.* (2001), who reported a 34% yield increase for grafts of *S. sisymbriifolium* rootstock (a wild tomato genotype) and 'BARI tomato-3' cultivar; but was much lower compared with that observed by Rivard and Louws (2006), who reported 104% increase for heirloom tomatoes on Hawaii 7996 rootstock. Goat apple

rootstocks impacted the least on tomato yields, under disease conditions, in comparison to other rootstocks tested. While, it out-yielded the control by up to 22.6% at Kapkatet site, it was inconsistent, recording a 5% yield decrease against control at Chesingoro site. This indicated the need to further evaluate the tolerance and potential usefulness of this rootstock, possibly under lower disease pressure.

In this study, yield differences between grafts and control plants under bacterial wilt disease conditions were less than expected, given the susceptibility of the scion variety. Other previous authors (Rashid *et al.* 2002; Aganon *et al.*, 2004) have reported higher percentage increase in yields as a result of grafting, dependent on the degree of infestation conditions which determine the survival rates among un-grafted control plants. Aganon *et al.* (2004) reported 332% and 240% yield increase when the susceptible cultivar, CL5915, was grafted on Hawaii 7996 and EG 203 (eggplant) rootstocks, respectively, under highly infested soil conditions, which had reduced the survival of the un-grafted control cultivar, to 64%, compared to 91.7% and 97.2% survival rates among grafts of the two rootstocks, respectively. Under similarly high infestation levels, Rashid *et al.* (2002), reported yield increase by 145%, for *S. torvum* rootstock grafted on 'BARI tomato-3' cultivar. In comparison, under less infested conditions in which the control cultivar had 26% bacterial wilt incidence, Ibrahim *et al.* (2001) found no increase in yields for grafts involving *S. torvum* and *S. integrifolium* rootstocks with 'BARI tomato-3' cultivar.

The relatively lower impact on tomato yields following grafting to disease tolerant rootstocks in the current study was caused by delayed disease development among inoculation treatments, affecting the plants late during the harvesting stage. Onset of wilting was not observed until 14 WAT, about six weeks after onset of harvesting. PDI values also indicated that the disease progressed gradually during the stage between 14 to 16 WAT. This suggests that environmental conditions around the root system of inoculated plants may have affected pathogen survival, resulting in delayed disease development.

Scientists have evaluated rootstocks to identify vigorous ones, which enhance yields in absence of disease pressure. It has been reported that interaction between cultivars and

suitable rootstocks result in high vigor of root systems, leading to increased water and nutrient uptake (Oda, 1995; Ruiz *et al.* 1996; Fernandez-Garcia *et al.* 2002; Kacjan-Marsic and Osvarld, 2004; Leonardi and Giuffrida, 2008), shoot growth promotion (Lee, 1994), and increased rates of photosynthesis (Matsuzoe *et al.* 1993), hence improving the yield performance of the scions. On the other hand, reduced growth and yields have been reported among grafts, where the rootstock was not suitably compatible with the scion (Romano and Paratore, 2001; Lopes *et al.* 2006). In the current study, Grafting to wild *S. aethiopicum* and DB3 rootstocks reduced scion yields by up to 20-23%. However, plant growth rates and yields of wild *S. aethiopicum* and DB3 grafts were not significantly different in comparison to un-grafted 'Anna F1' control plants, in absence of bacterial wilt disease inoculation. These findings therefore indicated that the two rootstocks were suitably compatible with the scion cultivar, but their effect as vigorous rootstocks was not apparent. The findings on these African eggplants generally agree with previous findings, which have indicated that eggplants rootstocks, while being highly bacterial wilt disease resistant, were typically not vigorous (Matsuzoe *et al.* 1993).

Non-inoculated wild tomato and goat apple grafts recorded higher yield reductions of up to 32-33%, in comparison to control plants. The lower yields observed for wild tomato grafts, in absence of disease conditions contrast with previous findings which showed that tomato genotypes rootstocks promoted yields. Khah *et al.* (2006), observed 32.5% and 12.8% increase in yields for 'Big Red' cultivar grafted on 'He-man' and 'Primavera' rootstocks, respectively, under greenhouse. Mohammed *et al.* (2009) observed 21% yield increase for 'Cecilia F1' grafted on 'Beaufort' rootstock; while Qaryouti *et al.* (2005) observed 16-38% yield increase on 'Cecilia' grafted on 'He-man' and 'Spirit', respectively. Similarly, yield increase of 11.5-17.5%, from grafts of 'Beril F1' cultivar with 'He-man' and 'Spirit' rootstocks, observed by Turkmen *et al.* (2010). Yield reduction among wild tomato grafts were not significant ( $p > 0.05$ ) at Chesingoro site, suggesting suitable graft compatibility with the scion variety, but significantly ( $p < 0.05$ ) lower yields were obtained at Kapkatet site. The lower yield performance of wild tomato grafts recorded at Kapkatet site indicated this rootstock was less vigorous compared to the hybrid scion variety. Previous authors have reported that interactions involving less vigorous rootstocks resulted in less vigorous

root systems hence reduced the reduced water and nutrient uptake leading to low yields in grafts (Ruiz *et al.* 1997; Fernandez-Garcia *et al.* 2002; Kacjan-Marsic and Osvarld, 2004; Leonardi and Giuffrida, 2008). The findings on goat apple grafts similarly suggested that this rootstock ranked as the least vigorous with respect to interaction with the scion variety, among the rootstocks tested.

This study revealed the main effects of grafting on yield related attributes that would be of great interest to growers. Grafting on any of the rootstocks did not impact on the time to onset of initial harvesting, which is normally obtained from the first truss, as an early yield. This result slightly contrasted with that of Ibrahim *et al.* (2001) who found a delay of only 4 days among grafts of *S. torvum*, *S. sysymbriifolium*, and *S. indicum*, in comparison to un-grafted 'BARI tomato 3' control, which they attributed to grafting shock. However, it should be noted that in this study, raising of seedlings were synchronized such that transplanting of the un-grafted control cultivar was done simultaneously with graft seedlings which were already healed and well taken, which possibly eliminated growth differences due to grafting shock. This finding is important to growers who are implementing production programs for a scheduled market. Under disease conditions, grafts achieved a higher number of clusters (fruit trusses), total fruit set and number of fruits per cluster than un-grafted control plants. Similar findings were reported by Lee (1994), and by Rashid *et al.* (2002). The results on cluster and fruit setting indicated that all the rootstocks helped to maintain normal reproductive development in 'Anna F1', when challenged by disease.

In this study, grafts tended to attain lower total fruit yield per plant, producing greater numbers of fruits having lower individual fruit weight, and slightly reduced fruit size, compared to the un-grafted non-inoculated control plants. Grafting effects on the yield attributes examined varied with rootstock used. This observation agrees with other authors who have observed varied correlations between fruit numbers, fruit sizes, and individual fruit weights with total plant yields. While Pogonyi *et al.* (2005) stated that yield increase in tomato was typically a result of increased fruit size, Passam *et al.* (2005) reported that both increased number and bigger fruit sizes contributed to improved yields among

eggplants grafted on tomato rootstocks. Turkmen *et al.* (2010) found that ‘Beril F1’ cultivar grafted on ‘He-man’ rootstock (tomato) produced a larger number of fruits, having smaller fruit size and lower individual fruit weight, but which amounted to improved total fruit yields per plant, in comparison to control. Conversely, Aganon *et al.* (2004), reported that, while higher numbers of fruits were obtained from grafts of ‘Apollo’ on both Hawaii 7996 and EG 203 rootstock, compared to those from CL5915 cultivar on both rootstocks, the total yield per plant were higher for the CL5915 graft combinations, due to larger fruit sizes and greater individual fruit weights. Fruit size and weight are critical quality parameters which affect marketability of produce. Goat apple and wild *S. aethiopicum* and grafts showed the strongest tendency to produce higher numbers of smaller and lighter fruits, compared to un-grafted ‘Anna F1’ control plants. Therefore, this will require growers to integrate optimum crop husbandry practices to minimize production of small sized fruits, and to channel small sized fruits to appropriate markets.

In this study, there was a narrow variation in fruit pulp pH among grafts and control plants. This finding agree with previous observations by Khah *et al.* (2006) who reported no difference among grafts of ‘Big red’ cultivar to ‘He-man’ and ‘Primavera’ rootstocks. However, other authors found that grafting affected tomato fruit pH, depending on the rootstock used (Ibrahim *et al.* 2001; Turkmen *et al.* 2010).

According to the study, grafting on any of the rootstocks did not significantly affect Brix value of fruits. This is in agreement with other previous reports that grafting did not influence this fruit quality parameter (Leoni *et al.* 1990; Romano and Paratore, 2001). However, previous reports on grafting effects on Brix are inconsistent and vary with crop type. For example, Lee (1994) reported reduced brix levels on grafted melon, while in tomato, Magambo *et al.* (2002) reported significantly higher Brix among grafts *S. incanum* and *S. macrocarpon* with tomato variety ‘Marglobe’ in comparison to un-grafted plants. Increase in Brix has been attributed to increased nutrient uptake and translocation, mainly phosphorous, nitrogen, magnesium, and calcium associated with fruit quality in xylem tissues in grafts (Ruiz *et al.*, 1997; Traka-Mavriona *et al.* 2000).

Grafting may also lead to lower concentrations of soluble solids in fruits, hence decreased Brix values, where vigorous rootstocks are used, since they cause increased yields, leading to a decrease in the concentration of the main fruit components (Augustin *et al.*, 2002; Pogonyi *et al.* 2005). On the other hand, increase in brix quality could also be attributed to reduced water absorption, hence a state of water stress in grafted fruits, causing reduced fruit size, thereby increasing soluble solid concentration in fruits (Matsuzoe *et al.* 1996). The fact that the findings of this study indicated no significant variations in brix quality among non-inoculated plants, in comparison to control plants, therefore suggests that though the rootstocks could have influenced nutrient and water absorption and affected yields of wild tomato and goat apple grafts, the threshold to significantly influence fruit Brix values was not met. However, under bacterial wilt disease inoculation, the Brix value the un-grafted control tomato fruits was higher than for fruits of grafts at Chesingoro site. This was attributed to greater water stress due to higher disease effects on the control plants in comparison to grafts.

Both pH and Brix co-determine taste and flavor in ripe fruits. Brix measurements indicate the sum content of soluble sugars, mainly glucose and fructose which impart sweetness taste (65%); and organic acids, mainly citric and malic acids (13%), while pH measurements indicate the proportion of the organic acids, which impart sourness variation in fruits (Causse *et al.*, 2002; Balibrea *et al.*, 2006). The fact that pH was nearly constant across grafts indicates that this parameter had a lesser influence on fruit taste quality as compared to Brix values, comparing non inoculated plants. Thus, the higher the brix value, the better the sweetness of tomato fruits. On this basis, sweetness was most improved by grafting on goat apple, while wild *S. aethiopicum* grafted fruits had the least sweet taste, in comparison to non-grafted fruits. Conversely, the fact that there was no significant decrease in fruit Brix among all grafts, in comparison to non-inoculated ‘Anna F1’ control plants, leads to the conclusion that grafting on each rootstock had no effect on the taste and flavor quality of the tomato scion variety tested.



## CHAPTER 6

### CONCLUSIONS AND RECOMMENDATIONS

#### 6.1 Conclusions

Kenyan tomato production is increasingly changing into indoor systems, which include the small holder farmer greenhouses already in widespread use in Bureti district. With continuous production under tomato mono-crops, the need for farmers to have techniques for managing bacterial wilt and other soil-borne diseases so as to sustain yields is urgent and inevitable.

Grafting was shown to completely control bacterial wilt disease and to enhance yields, when wild *S. aethiopicum* and DB3 rootstocks were employed as rootstocks for ‘Anna F1’ tomato variety. Under disease conditions, the wild tomato rootstock genotype investigated impacted moderate bacterial wilt disease tolerance on the scion cultivar, and similarly maintained tomato yields. These findings indicated that host tolerance among the rootstocks genotypes could be used effectively against bacterial wilt disease of tomato in Bureti district, under greenhouse infestation conditions ranging from low or moderate (wild tomato) to severe (wild *S. aethiopicum* and DB3). Goat apple ranked the least tolerant rootstock, and showed inconsistent yields of inoculated grafts, hence its against bacterial wilt disease was not established.

While growers will be interested in using resistant rootstocks which avert crop losses in hotspots, they would prefer those which also maintain or even enhance yields and fruit quality, under little or no disease pressure. Under disease free conditions, grafts of wild *S. aethiopicum* and DB3 rootstocks achieved comparable yields as the un-grafted tomato control plants, while those of wild tomato and goat apple rootstocks were significantly reduced in one of the sites. Therefore it was concluded that, while grafting to each rootstock was useful under disease conditions, their usefulness as vigorous rootstocks, solely to enhance yields under disease free conditions, was not apparent in all cases.

Grafting in all cases had no significant influence on fruit set, number of ripe fruits picked, and number of clusters per plant, number of fruits set per cluster and time to onset of first harvesting. Also, grafting did not affect Internal fruit quality (pH and Brix) parameters were not affected. However, grafts tended to produce larger numbers of smaller and lighter fruits in comparison to un-grafted tomato control fruits. Wild *S. aethiopicum* and goat apple grafted fruits were most affected with respect to reduced fruit sizes.

This is the first study establishing the efficacy of grafting as a control measure against bacterial wilt disease affecting greenhouse tomato production in Kenya. If adopted, grafting could compensate for the lack of crop rotation and sustain yields under severe soil bacterial wilt infestation, while being an environmentally safe method. This technique offers the most suitable option to the use of methyl bromide fumigant, banned globally due to its damage to the ozone layer (Batchelor, 2001; MBTOC, 2006). This technique could also be applied as an integrated pest management (IPM) tool against a wide range of other soil soil-borne pests and disease affecting greenhouse tomato production in Kenya, since previous research have established multiple tolerance among wild solanaceous rootstocks against other serious soil borne pests of tomato, including root-knot nematode, Fusarium wilt and Verticillium wilt (Williams *et al.*, 1991; Besri, 2002; Black *et al.*, 2003; AVRDC, 2005). All these advantages should be considered in justifying the potential cost for growers using the grafted tomato seedlings in Kenya.

## **6.2 Recommendations**

1. African eggplant rootstocks (wild *S. aethiopicum* and DB3) which were shown to be highly tolerant are recommended to growers to control severe bacterial wilt infestation. Selection of these rootstocks might involve a trade off on fruit size and internal quality, but total yields would be enhanced.
2. Wild tomato rootstock is recommended to growers only under low or moderate disease conditions, also to help stabilize yields.

3. None of the rootstocks investigated is recommended for use solely as vigorous rootstock since their usefulness in enhancing yield of 'Anna F1' under disease free conditions was not apparent.
4. Growers are advised to integrate optimum husbandry practices to minimize production of small sized fruit, notably with respect to wild *S. aethiopicum* and goat apple grafted fruits, which were found to be the most affected. Since grafting is an environmentally safe method, small sized produce should alternatively be channeled to markets involving consumers of organic products.
5. Further research is recommended to investigate the usefulness of goat apple as a tomato rootstock against bacterial wilt disease, since this rootstock did not consistently increase yields of the scion variety across study sites.
6. Further research is recommended to confirm the specific race and biovar which was presumed in this study to be Race3 biovar2
7. Since in this study test plants were artificially inoculated, in previously sterilized potted media, further investigations under naturally infested bacterial wilt hotspot are warranted. This is in order to exclude the effect of pots and to examine tolerance under interaction with other soil-borne pathogens, such as nematodes and *Fusarium spp.*
8. Further research is also recommended to evaluate their efficacy in controlling other soil borne pests; including root-knot nematodes and Fusarium wilt of tomato, which are both serious greenhouse pathogens in Kenya.
9. Further research to identify other vigorous rootstocks that may potentially enhance yields even in absence of disease conditions is also recommended.
10. Economic evaluations and adaptive trials with farmers required to convince them to adopt tomato grafting technology are recommended

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**APPENDIX I: DETAILED RESULTS TABLES AND FIGURES**

**Appendix I-1: Observation of bacterial wilt disease effects on inoculated rootstocks compared to control**

Site	Type of rootstock	Disease incidence (PWP)			Disease severity (PDI)		
		Wk 14	Wk 16	Wk 18	Wk 14	Wk 16	Wk 18
Chesingoro □ □ □	Wild <i>S. aethiopicum</i>						
		0a	0b	0c	0a	0b	0b
	DB3	0a	0b	0c	0a	0b	0b
	Wild tomato	0a	0b	11.1c	0a	0b	7.8b
	Goat apple	0a	0b	44.4b	0a	0b	15.6b
	Un-grafted 'Anna F1'	11.1a	46.7a	100a	4.4a	34.2a	82.7a
	Tukey's range test MSD <sup>2</sup>	17.8	16.3	25.9	7.1	15.5	19.3
Kapkatet □ □	Wild <i>S. aethiopicum</i>						
		0b	0c	0b	0b	0b	0c
	DB3	0b	0c	0b	0b	0b	0c
	Wild tomato	0b	5.6bc	22.2b	0b	4.4b	14.4bc
	Goat apple	5.6ab	27.8ab	38.9b	3.3ab	13.3ab	21.1b
	Un-grafted 'Anna F1'	22.2a	44.4a	83.3a	16.7a	30a	54.4a
	Tukey's range test MSD	19.5	25.0	40.6	14.3	22.0	19.0

**PWP = Percentage wilted plants    PDI = Percentage disease index    Wk 14-18 = Growth stages in weeks after transplanting**

<sup>1</sup>Values followed by the same letter(s) in the same column are not significantly different, according to Tukey’s HSD range test (p≥0.05)

<sup>2</sup>MSD = Minimum Significant Difference, according to Tukey’s HSD range test

**Appendix I-2: Observation of bacterial wilt disease effects on inoculated grafted plants and tomato ‘Anna F1’ control**

Study site	Rootstock x Scion combination	Disease incidence (PWP)			Disease severity (PDI)		
		Wk 14	Wk 16	Wk 18	Wk 14	Wk 16	Wk 18

Chesingoro	Wild <i>S. aethiopicum</i> x Anna F1	0.0 a <sup>1</sup>	0.0 c	0.0 c	0.0 a <sup>1</sup>	0.0 b	0.0 c
	DB3 x ‘Anna F1’	0.0 a	0.0 c	0.0 c	0.0 a	0.0 b	0.0 c
	Wild tomato x ‘Anna F1’	0.0 a	5.6 c	11.1 bc	0.0 a	1.1 b	10.0 bc
	Goat apple x ‘Anna F1’	11.1 a	38.9 ab	44.4 b	11.1 a	37.8 a	40.0 b
	Un-grafted ‘Anna F1’ (control)	11.1 a	46.7 a	100.0 a	4.4 a	34.2 a	82.7 a
	Tukey’s range test MSD <sup>2</sup>	23.7	43.3	37.2	18.4	41.3	41.9
Kapkatet	Wild <i>S. aethiopicum</i> x Anna F1	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b
	DB3 x ‘Anna F1’	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b
	Wild tomato x ‘Anna F1’	0.0 b	5.6 b	11.1 b	0.0 b	4.4 b	7.8 b
	Goat apple x ‘Anna F1’	0.0 b	5.6 b	11.1 b	0.0 b	4.4 b	7.8 b
	Un-grafted ‘Anna F1’ (control)	22.2 a	44.4 a	83.3 a	16.7 a	30.0 a	54.4 a
	Tukey’s range test MSD <sup>2</sup>	17.8	15.1	20.8	13.5	20.2	11.6

**PWP = Percentage wilted plants    PDI = Percentage disease index    Wk = Week after transplanting observed**

<sup>1</sup>Values followed by the same letter(s) in the same column are not significantly different, according to Tukey’s HSD range test (p≥0.05)

<sup>2</sup>MSD = Minimum Significant Difference, according to Tukey’s HSD range test

### AppendixI-3: Comparison of plant height (cm) of rootstocks and tomato ‘Anna F1’ control at observed growth stages

Site	Rootstock	BW disease inoculation <sup>1</sup>	Time (Weeks after transplanting)									
			0	2	4	6	8	10	12	14	16	18
Chesingoro	Wild <i>S. aethiopicum</i>	NI	5.8c <sup>2</sup>	12.4c	36.3d	60.9bcd	78.0cd	86.3bcd	86.7cd	87.1b	91.1b	94.9 a

		I	6.8bc	14.1bc	35.6d	62.4bcd	79.3cd	88.7bcd	89.7cd	91.2b	92.3b	93.4 a
	DB3	NI	5.7c	12.6c	27.4d	45.1cd	65.4d	78.6cd	83.7d	87.6b	91.4b	96.4 a
		I	5.2c	11.2c	24.4d	38.7d	55.7d	71.7cd	78.2d	81.8b	86.5b	92.6 a
	Wild tomato	NI	12.8a	31.7a	51.9b	79.2b	104.9abc	119.6ab	137.1ab	143.4a	150.1a	157.0 b
		I	11.9a	26.3a	43.9bc	67.3bc	83.3bcd	100.0bc	115.1bc	126.2a	136.5a	144.1 b
	Goat apple	NI	15.0a	19.2a	28.3cd	36.8cd	46.9d	59.2d	71.6d	80.8b	91.7b	98.2 a
		I	16.1a	20.2a	29.1cd	37.9cd	46.1d	57.5d	66.4d	71.7b	76.7b	79.6 a
	Un-grafted 'Anna F1'	NI	13.4a	43.1a	70.9a	91.9a	120.7a	138.4a	145.0ab	148.6a	157.9a	164.8 b
		I	15.3a	46.4a	73.0a	93.1a	119.3a	141.3a	146.2a	147.1a	148.7a	149.9 b
		Minimum significant difference <sup>3</sup>	5.5	9.3	17.5	23.0	37.3	37.9	30.2	27.3	32.7	39.2
Kapkatet	Wild <i>S. aethiopicum</i>	NI	7.6bc	15.3cd	35.1d	66.8bc	96.2ab	105.8ab	112.1ab	118.6ab	127.2ab	136.3 a
		I	8.1bc	15.4cd	36.8cd	66.4bc	98.9a	108.1ab	112.7ab	120.2ab	129.5ab	136.1 a
	DB3	NI										
			7.0c	13.1d	25.7d	44.1cd	70.7bc	79.5bc	86.8bc	92.3bc	100.6bc	107.2 a
		I	6.9c	13.8cd	23.6d	38.7d	60.7c	67.4c	71.7c	77.8c	84.9c	94.0 b
	Wild tomato	NI	11.7ab	27.0b	50.8b	79.6ab	100.6a	110.7a	116.6ab	127.1a	135.8a	147.4 a
		I	12.9a	27.0b	49.5bc	75.0b	98.6a	108.7a	115.8ab	124.8a	131.5ab	142.8 a
	Goat apple	NI										
			15.1a	18.5cd	27.1d	37.1d	51.6c	58.8c	69.1c	81.1c	98.8bc	113.6 a
		I	14.8a	20.1c	28.3d	39.2d	53.0c	61.2c	65.5c	75.7c	86.8c	99.0 a
	Un-grafted 'Anna F1'	NI	15.6a	37.3a	70.8a	99.7a	118.3a	122.5a	126.7a	130.8a	136.3a	149.9a
		I	15.3a	39.1a	71.6a	102.4a	120.8a	125.3a	126.9a	127.6a	129.4ab	135.5b
		Minimum significant difference	4.9	6.8	13.7	24.0	25.8	28.6	31.5	30.3	33.9	38.8

**NI = Not inoculated with bacterial wilt (BW) disease    I = Inoculated with Bacterial wilt**

<sup>1</sup>Bacterial wilt disease inoculation was done at 3 WAT and repeated at 4WAT    <sup>2</sup>Values in each column followed by the same letter (s) are not significantly different, according to Tukey's HSD range test ( $p \geq 0.05$ ).    <sup>3</sup>Tukey's range test Minimum significant difference

**Appendix I-4: Plant height (cm) of grafted plants and un-grafted control at observed growth stages at Chesingoro site**

Site	Grafting rootstock used		BW <sup>1</sup> inoculation	Time (Weeks after transplanting)									
				0	2	4	6	8	10	12	14	16	18
Chesingoro	Wild <i>S. aethiopicum</i>	NI	40.1 a <sup>2</sup>	64.0a	89.3 a	103.8a	125.3a	154.3a	170.9 a	177.2 a	186.3 a	197.3a	
		I	40.0 a	62.4a	84.9ab	103.4a	124.8a	157.4a	161.9ab	168.4ab	179.7ab	186.8ab	
	DB3	NI	28.3 b	45.7bc	70.8bcd	93.6a	118.2a	143.9a	158.7ab	169.0ab	178.8ab	189.6a	
		I	31.9 b	52.3a	76.9abc	94.7a	119.8a	145.3a	152.7ab	158.7ab	164.6ab	169.2abc	
	Wild tomato	NI	26.3 b	44.8bcd	75.2abcd	97.3a	119.0a	146.5a	156.8ab	161.5ab	164.9ab	168.7abc	
		I	26.6 b	51.0b	75.1abcd	93.0a	118.2a	137.9a	139.3b	143.2 b	146.3 b	148.1c	
	Goat apple	NI	25.0 b	35.0d	61.5 d	91.6a	115.2a	140.2a	153.1ab	158.9ab	172.9ab	178.5abc	
		I	27.5 b	39.8cd	67.1cd	91.8a	114.2a	136.8a	141.8ab	143.5 b	148.4 b	150.8bc	
	Un-grafted 'Anna F1'	NI	13.4 c	43.1bcd	70.9bcd	91.9a	120.7a	138.4a	145.0ab	148.6ab	157.9ab	164.8abc	
		I	15.3 c	46.4bc	73.0bcd	93.1a	119.3a	141.3a	146.2ab	147.1ab	148.7 b	149.9bc	
Minimum significant difference <sup>3</sup>				7.8	10.1	15.2	15.6	20.3	24.4	31.1	33.1	36.3	37.6
Kapkatet	Wild <i>S. aethiopicum</i>	NI	40.8a	56.6a	85.7a	113.4a	140.2a	146.4a	151.0a	158.7a	169.2a	190.6a	
		I	41.6a	59.0a	86.9a	110.7a	135.1ab	140.2ab	144.3ab	151.4ab	165.7a	185.2a	
	DB3	NI	19.9c	26.0d	45.7d	74.1d	108.1c	119.8cd	126.2bc	133.6bc	139.1bcd	151.1bc	
		I	20.2c	28.6cd	48.9cd	76.8bd	105.4c	113.9d	118.9c	125.6c	135.2cd	150.7bc	
	Wild tomato	NI	25.1bc	39.8b	59.6cd	83.7bcd	113.7bc	120.9cd	127.2bc	133.7bc	142bcd	152.8bc	
		I	20.7c	31.9bcd	52.1cd	79.9cd	108.3c	119.2cd	122.3c	128.0c	133.5cd	141.2bc	
	Goat apple	NI	27.8b	38.5b	65.1b	96.4abc	124.7abc	132.4abcd	135.4abc	142.2abc	156ab	168.1ab	
		I	31.2b	40.4b	63.8b	96.2abc	126.7abc	135.9abc	138.4abc	143.5abc	151.9abc	166.6ab	
	Un-grafted 'Anna F1'	NI	15.6d	37.3bc	70.8ab	99.7ab	118.3bc	122.5bcd	126.7bc	130.8bc	136.3cd	149.9bc	
		I	15.3d	39.1b	71.6ab	102.4a	120.8abc	125.3bcd	126.9bc	127.6c	129.4d	135.5c	
Minimum significant difference				6.2	9.3	16.7	18.6	21.6	19.1	20.8	22.0	20.3	29.5

**NI = Not inoculated with bacterial wilt (BW) disease    I = Inoculated with Bacterial wilt**

<sup>1</sup>Bacterial wilt disease inoculation was done at 3 WAT and repeated at 4WAT    <sup>2</sup>Values in each column followed by the same letter (s) are not significantly different, according to Tukey's HSD range test ( $p \geq 0.05$ ).    <sup>3</sup>Tukey's range test Minimum significant difference

**Appendix I-5: Plant growth rates (cm/ fortnight) of grafts compared to ‘Anna F1’ control**

Site	Grafting rootstock used	BW disease <sup>1</sup> inoculation	Time (Weeks after transplanting)									
			2	4	6	8	10	12	14	16	18	
Chesingoro	Wild <i>S. aethiopicum</i>	NI	23.9bc <sup>2</sup>	25.3a	14.6b	21.4a	29.0a	16.7a	6.2ab	9.2a	10.9a	
		I	22.4cd	22.5a	18.5b	21.4a	32.6a	4.4ab	6.6ab	11.2ab	7.2a	
	DB3	NI	17.4de	25.1a	22.7ab	24.7a	25.7a	14.8a	10.3a	9.8abc	10.8a	
		I	20.4cd	24.6a	17.8b	25.1a	25.5a	7.4ab	6.1ab	5.8abc	4.7a	
	Wild tomato	NI	18.5cde	30.4a	22.1ab	21.7a	27.6a	10.3ab	4.7ab	3.4bc	3.8a	
		I	24.5bc	24.1a	17.9b	25.2a	19.7a	1.4b	3.9ab	3.1bc	1.8a	
	Goat apple	NI	10.0f	26.5a	30.1a	23.6a	25.0a	12.9ab	5.9ab	14.0abc	5.6a	
		I	12.3ef	27.3a	24.8ab	22.3a	22.6a	5.0ab	1.7b	4.9abc	2.3a	
	Un-grafted ‘Anna F1’	NI	29.7ab	27.8a	20.9ab	28.8a	17.7a	6.6ab	3.6ab	9.2abc	6.9a	
		I	31.2a	26.6a	20.1ab	26.2a	21.9a	4.9ab	0.9b	1.7c	1.1a	
		Minimum significant difference <sup>3</sup>	6.2	11.9	10.5	13.0	17.4	11.8	7.7	9.5	10.1	
	Kapkatet	Wild <i>S. aethiopicum</i>	NI	15.8abc	29.1ab	27.7a	26.8ab	6.2a	4.6a	7.7a	10.5ab	21.4a
			I	17.4ab	27.9ab	23.8a	24.4ab	5.1a	4.1a	7.1ab	14.3a	19.5ab
DB3		NI	6.1c	19.7b	28.4a	34a	11.7a	6.4a	7.4a	5.5bc	12ab	
		I	8.4bc	20.3b	27.9a	28.6ab	8.5a	5a	6.7ab	9.6ab	15.5ab	
Wild tomato		NI	14.7abc	19.8b	24.1a	30.0ab	7.2a	6.3a	6.5ab	8.3abc	10.8ab	
		I	11.2bc	20.2b	27.8a	28.4ab	10.9a	3.1a	5.7ab	5.5bc	7.7ab	
Goat apple		NI	10.7bc	26.6ab	31.3a	28.3ab	7.7a	3a	6.8ab	13.8a	12.1ab	
		I	9.2bc	23.4ab	32.4a	30.5ab	9.2a	2.5a	5.1ab	8.4abc	14.7ab	
Un-grafted ‘Anna F1’		NI	21.7a	33.5a	28.9a	18.6b	4.2a	4.2a	4.1ab	5.5bc	13.6ab	
		I	23.8a	32.5a	30.8a	18.4b	4.5a	1.6a	0.7b	1.8c	6.1b	
		Minimum significant difference	10.3	11.4	14.4	12.7	8.9	6.2a	6.4	7.1	15.4	

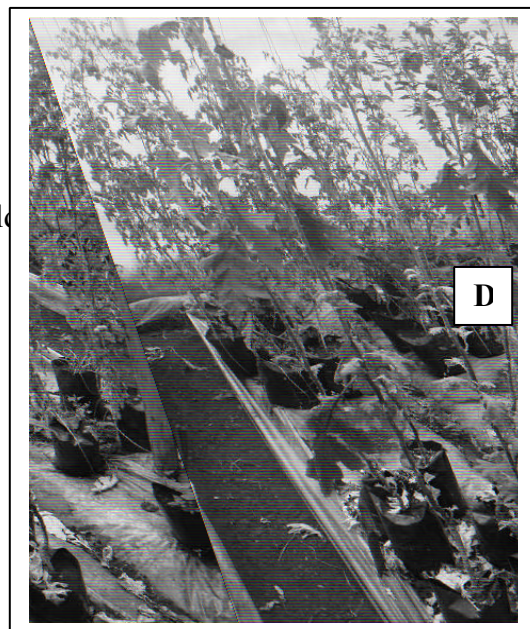
**NI = Not inoculated with bacterial wilt (BW) disease    I = Inoculated with Bacterial wilt**



<sup>1</sup>Bacterial wilt disease inoculation was done at 3 WAT and repeated at 4WAT    <sup>2</sup>Values in each column followed by the same letter (s) are not significantly different, according to Tukey's HSD range test ( $p \geq 0.05$ ).    <sup>3</sup>Tukey's range test Minimum significant difference

## APPENDIX II: RESEARCH PROGRAM PHOTOGRAPHS

### 1. Types of rootstocks screened for bacterial wilt tolerance



Wild

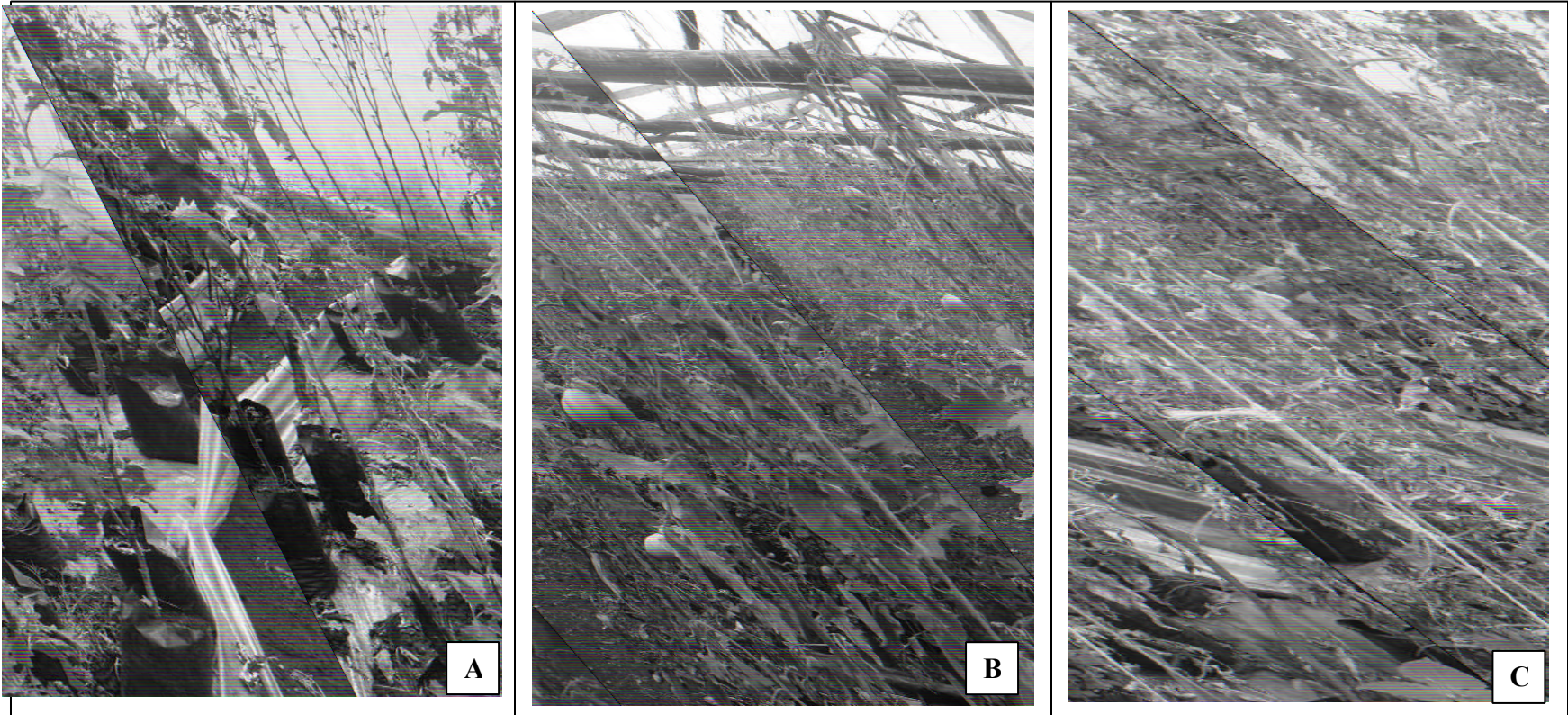
wild

C

10



## 2. Observation of bacterial wilt disease treatment effects



**Appendix II-2:** Observation of wilting symptoms. Plants were also stunted in growth compared to control rootstocks; **C:** Dead and collapsed un-grafted

apple rootstock plant (note the wilted goat apple observed on tomato grafted on goat apple complete wilting).

**APPENDIX III: ANOVA TABLES**

**Appendix III -1: ANOVA table for Percent wilted plants (PWP) for rootstocks at Chesingoro and Kapkatet sites**

**Rootstocks PWP at Chesingoro site**

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**The GLM Procedure**

Class Level Information

Class	Levels	Values	
Treatment	10	1 2 3 4 5 6 7 8 17 18	
Block	3	1 2 3	
Number of Observations Used			30

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**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	407.407407	37.037037	1.00	0.4825
Error	18	666.666667	37.037037		
Corrected Total	29	1074.074074			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.379310	547.7226	6.085806	1.111111

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	333.3333333	37.0370370	1.00	0.4742
Block	2	74.0740741	37.0370370	1.00	0.3874

---

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	5942.222222	540.202020	17.36	<.0001
Error	18	560.000000	31.111111		
Corrected Total	29	6502.222222			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.913876	119.5229	5.577734	4.666667

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	5880.000000	653.333333	21.00	<.0001
Block	2	62.222222	31.111111	1.00	0.3874

---

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	29111.11111	2646.46465	33.85	<.0001
Error	18	1407.40741	78.18930		
Corrected Total	29	30518.51852			

R-Square	Coeff Var	Root MSE	Wk_18 Mean
0.953883	56.84446	8.842471	15.55556

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	29037.03704	3226.33745	41.26	<.0001
Block	2	74.07407	37.03704	0.47	0.6302

---

**(b): Rootstocks PWP at Kapkatet site**

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The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	1 2 3 4 5 6 7 8 17 18
Block	3	1 2 3
Number of Observations Used		30

---

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1472.222222	133.838384	3.03	0.0181
Error	18	796.296296	44.238683		
Corrected Total	29	2268.518519			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.648980	239.4438	6.651217	2.777778

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	1342.592593	149.176955	3.37	0.0135
Block	2	129.629630	64.814815	1.47	0.2573

---

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	6870.370370	624.579125	8.55	<.0001
Error	18	1314.814815	73.045267		
Corrected Total	29	8185.185185			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.839367	109.8855	8.546652	7.777778

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	9	6518.518519	724.279835	9.92	<.0001
Block	2	351.851852	175.925926	2.41	0.1183

---

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	21388.88889	1944.44444	10.11	<.0001
Error	18	3462.96296	192.38683		
Corrected Total	29	24851.85185			

R-Square	Coeff Var	Root MSE	Wk_18 Mean
0.860656	96.02556	13.87036	14.44444

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	20592.59259	2288.06584	11.89	<.0001
Block	2	796.29630	398.14815	2.07	0.1552

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**Appendix III-2 ANOVA table for rootstock Percentage Disease Incidence (PDI) at Chesingoro and Kapkatet sites**

**(a): Rootstocks PDI at Chesingoro site**

-----  
 The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	1 2 3 4 5 6 7 8 17 18
Block	3	1 2 3
Number of Observations Used		30

-----  
**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	65.1851852	5.9259259	1.00	0.4825
Error	18	106.6666667	5.9259259		
Corrected Total	29	171.8518519			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.379310	547.7226	2.434322	0.444444

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	53.33333333	5.92592593	1.00	0.4742
Block	2	11.85185185	5.92592593	1.00	0.3874

-----  
**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3218.162963	292.560269	10.44	<.0001
Error	18	504.266667	28.014815		
Corrected Total	29	3722.429630			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.864533	154.6627	5.292902	3.422222

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	3162.133333	351.348148	12.54	<.0001
Block	2	56.029630	28.014815	1.00	0.3874

-----

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	18061.76296	1641.97845	37.45	<.0001
Error	18	789.21481	43.84527		
Corrected Total	29	18850.97778			

R-Square    Coeff Var    Root MSE    Wk\_18 Mean  
0.958134    62.46770    6.621576    10.60000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	18037.94074	2004.21564	45.71	<.0001
Block	2	23.82222	11.91111	0.27	0.7652

---

**(b):Rootstocks PDI at Kapkatet site**

---

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	1 2 3 4 5 6 7 8 17 18
Block	3	1 2 3
Number of Observations Used		30

---

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	808.888889	73.535354	3.10	0.0162
Error	18	426.666667	23.703704		
Corrected Total	29	1235.555556			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.654676	243.4322	4.868645	2.000000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	746.6666667	82.9629630	3.50	0.0114
Block	2	62.2222222	31.1111111	1.31	0.2937

---

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2684.074074	244.006734	4.31	0.0031
Error	18	1020.000000	56.666667		
Corrected Total	29	3704.074074			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.724628	157.5571	7.527727	4.777778

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2607.777778	289.753086	5.11	0.0016
Block	2	76.296296	38.148148	0.67	0.5225

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	8641.111111	785.555556	18.55	<.0001
Error	18	762.222222	42.345679		
Corrected Total	29	9403.333333			

R-Square    Coeff Var    Root MSE    Wk\_18 Mean  
0.918941    72.30395    6.507356    9.000000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	8425.555556	936.172840	22.11	<.0001
Block	2	215.555556	107.777778	2.55	0.1063

---

**Appendix Table III – 3: ANOVA table for PWP for grafts at Chesingoro and Kapkatet sites**

**(a):Grafts PWP at Chesingoro site**

---

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

---

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	888.888889	80.808081	1.23	0.3378
Error	18	1185.185185	65.843621		
Corrected Total	29	2074.074074			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.428571	365.1484	8.114408	2.222222

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	592.5925926	65.8436214	1.00	0.4742
Block	2	296.2962963	148.1481481	2.25	0.1342

---

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	9064.444444	824.04040	3.77	0.0063
Error	18	3934.07407	218.55967		
Corrected Total	29	12998.51852			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.697344	162.2608	14.78376	9.111111

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	8672.592593	963.621399	4.41	0.0036
Block	2	391.851852	195.925926	0.90	0.4255

---

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	29277.77778	2661.61616	16.48	<.0001
Error	18	2907.40741	161.52263		
Corrected Total	29	32185.18519			

R-Square	Coeff Var	Root MSE	Wk_18 Mean
0.909666	81.70171	12.70916	15.55556

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	29037.03704	3226.33745	19.97	<.0001
Block	2	240.74074	120.37037	0.75	0.4887

---

**(b):Grafts PWPat Kapkatet site**

-----  
The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

-----

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1407.407407	127.946128	3.45	0.0097
Error	18	666.666667	37.037037		
Corrected Total	29	2074.074074			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.678571	273.8613	6.085806	2.222222

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	1333.333333	148.148148	4.00	0.0060
Block	2	74.074074	37.037037	1.00	0.3874

-----

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	5259.259259	478.114478	17.87	<.0001
Error	18	481.481481	26.748971		
Corrected Total	29	5740.740741			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.916129	93.09493	5.171941	5.555556

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	5185.185185	576.131687	21.54	<.0001
Block	2	74.074074	37.037037	1.38	0.2758

-----

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	18250.00000	1659.09091	32.91	<.0001
Error	18	907.40741	50.41152		
Corrected Total	29	19157.40741			

R-Square    Coeff Var    Root MSE    Wk\_18 Mean  
0.952634    67.26417    7.100107    10.55556

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	18231.48148	2025.72016	40.18	<.0001
Block	2	18.51852	9.25926	0.18	0.8337

---



**Appendix III- 4 ANOVA table for Percent Disease Index (PDI) for grafts at Chesingoro and Kapkatet sites**

**(a): PDI for grafts at Chesingoro site**

-----  
 The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

-----

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	502.222222	45.656566	1.15	0.3819
Error	18	714.074074	39.670782		
Corrected Total	29	1216.296296			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.412911	404.9019	6.298475	1.555556

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	357.0370370	39.6707819	1.00	0.4742
Block	2	145.1851852	72.5925926	1.83	0.1890

-----

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	6406.681481	582.425589	2.92	0.0211
Error	18	3585.748148	199.208230		
Corrected Total	29	9992.429630			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.641154	193.0502	14.11411	7.311111

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	6195.096296	688.344033	3.46	0.0121
Block	2	211.585185	105.792593	0.53	0.5969

---

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	20633.02222	1875.72929	9.16	<.0001
Error	18	686.40000	204.80000		
Corrected Total	29	24319.42222			

R-Square	Coeff Var	Root MSE	Wk_18 Mean
0.848417	107.8706	14.31084	13.26667

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	9	20321.20000	2257.91111	11.02	<.0001
Block	2	311.82222	155.91111	0.76	0.4815

---

**(b): PDI for grafts at Kapkatet site**

-----  
The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

-----

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	792.222222	72.020202	3.41	0.0103
Error	18	380.000000	21.111111		
Corrected Total	29	1172.222222			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.675829	275.6810	4.594683	1.666667

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	750.000000	83.3333333	3.95	0.0064
Block	2	42.2222222	21.1111111	1.00	0.3874

-----

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2436.666667	221.515152	4.67	0.0020
Error	18	854.074074	47.448560		
Corrected Total	29	3290.740741			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.740461	177.1275	6.888291	3.888889

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2364.814815	262.757202	5.54	0.0010
Block	2	71.851852	35.925926	0.76	0.4834

---

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	7814.444444	710.404040	45.31	<.0001
Error	18	282.222222	15.679012		
Corrected Total	29	8096.666667			

R-Square	Coeff Var	Root MSE	Wk_18 Mean
0.965143	56.56676	3.959673	7.000000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	7785.555556	865.061728	55.17	<.0001
Block	2	28.888889	14.444444	0.92	0.4160

---

**Appendix III- 5: ANOVA table for Rootstock plant height (cm) at Chesingoro and Kapkatet sites**

**(a):Rootstock Plant height (cm) at Chesingoro site**

**The GLM Procedure**

Class Level Information

Class	Levels	Values
Treatment	10	1 2 3 4 5 6 7 8 17 18
Block	3	1 2 3
Number of Observations Used		30

**Dependent Variable: Wk 0**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	534.0243056	48.5476641	13.73	<.0001
Error	18	63.6657407	3.5369856		
Corrected Total	9	597.6900463			
	R-Square	Coeff Var	Root MSE	Wk_0 Mean	
	0.893480	17.43620	1.880688	10.78611	

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	531.1668981	59.0185442	16.69	<.0001
Block	2	2.8574074	1.4287037	0.40	0.6736

**Dependent Variable: Wk 2**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	4540.384028	412.762184	41.29	<.0001
Error	18	179.931944	9.996219		
Corrected Total	29	4720.315972			

R-Square	Coeff Var	Root MSE	Wk_2 Mean
0.961881	13.33573	3.161680	23.70833

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	4496.815972	499.646219	49.98	<.0001
Block	2	43.568056	21.784028	2.18	0.1421

---

**Dependent Variable: Wk 4**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	8724.776852	793.161532	22.21	<.0001
Error	18	642.872222	35.715123		
Corrected Total	29	9367.649074			

R-Square	Coeff Var	Root MSE	Wk_4 Mean
0.931373	14.19715	5.976213	42.09444

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	8548.130556	949.792284	26.59	<.0001
Block	2	176.646296	88.323148	2.47	0.1125

---

**Dependent Variable: Wk 6**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	13156.27152	1196.02468	19.31	<.0001
Error	18	1115.12511	61.95140		
Corrected Total	29	14271.39663			

R-Square	Coeff Var	Root MSE	Wk_6 Mean
0.921863	12.83047	7.870921	61.34556

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	12668.70700	1407.63411	22.72	<.0001
Block	2	487.56452	243.78226	3.94	0.0382

---

**Dependent Variable: Wk 8**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	21483.24985	1953.02271	12.04	<.0001
Error	18	2918.90215	162.16123		
Corrected Total	29	24402.15200			

R-Square    Coeff Var    Root MSE    Wk\_8 Mean  
0.880383    15.92313    12.73425    79.97333

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	20672.44385	2296.93821	14.16	<.0001
Block	2	810.80600	405.40300	2.50	0.1101

---

**Dependent Variable: Wk 10**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	25643.44977	2331.22271	13.94	<.0001
Error	18	3010.75139	167.26397		
Corrected Total	29	28654.20116			

R-Square    Coeff Var    Root MSE    Wk\_10 Mean  
0.894928    13.74192    12.93306    94.11389

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	24785.94653	2753.99406	16.46	<.0001
Block	2	857.50324	428.75162	2.56	0.1048

---

**Dependent Variable: Wk 12**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	26897.69370	2445.24488	22.92	<.0001
Error	18	1920.34126	106.68563		
Corrected Total	29	28818.03496			

R-Square    Coeff Var    Root MSE    Wk\_12 Mean  
0.933363    10.13010    10.32887    101.9622

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	26050.85941	2894.53993	27.13	<.0001
Block	2	846.83430	423.41715	3.97	0.0373

---

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	26897.36436	2445.21494	28.20	<.0001
Error	18	1560.88383	86.71577		
Corrected Total	29	2 8458.24819			

R-Square    Coeff Var    Root MSE    Wk\_14 Mean  
0.945152    8.740365    9.312130    106.5417

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	25849.34986	2872.14998	33.12	<.0001
Block	2	1048.01450	524.00725	6.04	0.0098

---

**Dependent Variable: Wk 16**

Sum of

Source	DF	Squares	Mean Square	F Value	Pr > F
Model	11	28332.15336	2575.65031	20.70	<.0001
Error	18	2239.49202	124.41622		
Corrected Total	29	30571.64538			

R-Square    Coeff Var    Root MSE    Wk\_16 Mean  
0.926746    9.933634    11.15420    112.2872

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	27191.06223	3021.22914	24.28	<.0001
Block	2	1141.09113	570.54556	4.59	0.0246

---



**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	29622.64026	2692.96730	15.00	<.0001
Error	18	3230.91696	179.49539		
Corrected Total	29	32853.55722			

R-Square	Coeff Var	Root MSE	Wk_18 Mean
0.901657	11.44278	13.39759	117.0833

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	9	28535.48537	3170.60949	17.66	<.0001
Block	2	1087.15489	543.57744	3.03	0.0735

---

**(b): Plant height (cm)for rootstocks at Kapkatet site**

---

**The GLM Procedure**

Class Level Information

Class	Levels	Values
Treatment	10	1 2 3 4 5 6 7 8 17 18
Block	3	1 2 3
Number of Observations Used		30

---

**Dependent Variable: Wk0**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	375.3190648	34.1199150	13.91	<.0001
Error	18	44.1557593	2.4530977		
Corrected Total	29	419.4748241			

R-Square	Coeff Var	Root MSE	Wk0 Mean
0.894736	13.60827	1.566237	11.50944

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	9	374.9231574	41.6581286	16.98	<.0001
Block	2	0.3959074	0.1979537	0.08	0.9228

---

**Dependent Variable: Wk2**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2495.022222	226.820202	41.44	<.0001
Error	18	98.529630	5.473868		
Corrected Total	29	2593.551852			

R-Square	Coeff Var	Root MSE	Wk2 Mean
0.962010	10.32696	2.339630	22.65556

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2467.792593	274.199177	50.09	<.0001
Block	2	27.229630	13.614815	2.49	0.1112

---

**Dependent Variable: Wk 4**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	8898.676852	808.970623	37.09	<.0001
Error	18	392.587037	21.810391		
Corrected Total	29	9291.263889			

R-Square    Coeff Var    Root MSE    Wk\_4 Mean  
0.957747    11.14153    4.670160    41.91667

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	8785.837963	976.204218	44.76	<.0001
Block	2	112.838889	56.419444	2.59	0.1029

---

**Dependent Variable: Wk 6**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	17164.98704	1560.45337	23.06	<.0001
Error	18	1218.10926	67.67274		
Corrected Total	29	18383.09630			

R-Square    Coeff Var    Root MSE    Wk\_6 Mean  
0.933738    12.67324    8.226344    64.91111

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	16474.09630	1830.45514	27.05	<.0001
Block	2	690.89074	345.44537	5.10	0.0175

---

**Dependent Variable: Wk 8**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	19566.68704	1778.78973	22.94	<.0001
Error	18	1395.52037	77.52891		
Corrected Total	29	20962.20741			

R-Square    Coeff Var    Root MSE    Wk\_8 Mean  
0.933427    10.12980    8.805050    86.92222

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	18104.76296	2011.64033	25.95	<.0001
Block	2	1461.92407	730.96204	9.43	0.0016

---

**Dependent Variable: Wk 10**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	19189.29800	1744.48164	18.25	<.0001
Error	18	1720.36067	95.57559		
Corrected Total	29	20909.65867			

R-Square    Coeff Var    Root MSE    Wk\_10 Mean  
0.917724    10.31325    9.776277    94.79333

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	17532.60978	1948.06775	20.38	<.0001
Block	2	1656.68822	828.34411	8.67	0.0023

**Dependent Variable: Wk 12**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	18207.58319	1655.23484	14.29	<.0001
Error	18	2084.68956	115.81609		
Corrected Total	29	20292.27274			

R-Square    Coeff Var    Root MSE    Wk\_12 Mean  
0.897267    10.71939    10.76179    100.3956

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	16150.30978	1794.47886	15.49	<.0001
Block	2	2057.27341	1028.63670	8.88	0.0021

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	16361.84367	1487.44033	13.91	<.0001
Error	18	1924.75933	106.93107		
Corrected Total	29	18286.60300			

R-Square    Coeff Var    Root MSE    Wk\_14 Mean  
0.894745    9.610659    10.34075    107.5967

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	14191.31411	1576.81268	14.75	<.0001
Block	2	2170.52956	1085.26478	10.15	0.0011

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	14013.43315	1273.94847	9.52	<.0001
Error	18	2408.30704	133.79484		
Corrected Total	29	16421.74019			

R-Square    Coeff Var    Root MSE    Wk\_16 Mean  
0.853346    9.965323    11.56697    116.0722

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	11643.38685	1293.70965	9.67	<.0001
Block	2	2370.04630	1185.02315	8.86	0.0021

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	13756.63406	1250.60310	7.10	0.0002
Error	18	3169.24909	176.06939		
Corrected Total	29	16925.88316			

R-Square    Coeff Var    Root MSE    Wk\_18 Mean  
0.812757    10.51653    13.26911    126.1739

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	11616.62482	1290.73609	7.33	0.0002
Block	2	2140.00924	1070.00462	6.08	0.0096

---

**Appendix III – 6: ANOVA table for plant height for grafts at Chesingoro and Kapkatet sites**

**(a): Grafts plant height (cm) at Chesingoro site**

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 The GLM Procedure

Class Level Information

Class      Levels    Values

Treatment    10    9 10 11 12 13 14 15 16 17 18

Block            3    1 2 3

Number of Observations Used            30

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**Dependent Variable: Wk 0**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2091.647917	190.149811	27.03	<.0001
Error	18	126.625463	7.034748		
Corrected Total	29	2218.273380			

R-Square    Coeff Var    Root MSE    Wk\_0 Mean  
 0.942917    9.667223    2.652310    27.43611

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2071.305787	230.145087	32.72	<.0001
Block	2	20.342130	10.171065	1.45	0.2616

-----

**Dependent Variable: Wk 2**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2395.721259	217.792842	18.36	<.0001
Error	18	213.565259	11.864737		
Corrected Total	29	2609.286519			

R-Square    Coeff Var    Root MSE    Wk\_2 Mean  
 0.918152    7.106667    3.444523    48.46889

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2305.490963	256.165663	21.59	<.0001
Block	2	90.230296	45.115148	3.80	0.0419

---

**Dependent Variable: Wk 4**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2038.482250	185.316568	6.91	0.0002
Error	18	483.078685	26.837705		
Corrected Total	29	2521.560935			

R-Square	Coeff Var	Root MSE	Wk_4 Mean
0.808421	6.955627	5.180512	74.47944

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	1757.807787	195.311976	7.28	0.0002
Block	2	280.674463	140.337231	5.23	0.0162

---

**Dependent Variable: Wk 6**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	881.325407	80.120492	2.82	0.0246
Error	18	510.873111	28.381840		
Corrected Total	29	1392.198519			

R-Square	Coeff Var	Root MSE	Wk_6 Mean
0.633046	5.583040	5.327461	95.42222

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	581.0044444	64.5560494	2.27	0.0659
Block	2	300.3209630	150.1604815	5.29	0.0156

---

**Dependent Variable: Wk 8**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	511.495843	46.499622	0.97	0.5054
Error	18	864.013315	48.000740		
Corrected Total	29	1375.509157			

R-Square	Coeff Var	Root MSE	Wk_8 Mean
0.371859	5.799295	6.928257	119.4672

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	342.4630463	38.0514496	0.79	0.6272
Block	2	169.0327963	84.5163981	1.76	0.2003

---

**Dependent Variable: Wk 10**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1594.290148	144.935468	2.09	0.0802
Error	18	1250.571926	69.476218		
Corrected Total	29	2844.862074			

R-Square	Coeff Var	Root MSE	Wk_10 Mean
0.560410	5.784433	8.335240	144.0978

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	1270.066519	141.118502	2.03	0.0959
Block	2	324.223630	162.111815	2.33	0.1256

**Dependent Variable: Wk 12**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3078.768620	279.888056	2.48	0.0423
Error	18	2033.408315	112.967129		
Corrected Total	29	5112.176935			

R-Square	Coeff Var	Root MSE	Wk_12 Mean
0.602242	6.958648	10.62860	152.7394

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2622.391935	291.376882	2.58	0.0416
Block	2	456.376685	228.188343	2.02	0.1616

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	4269.425630	388.129603	3.04	0.0176
Error	18	2294.840148	127.491119		
Corrected Total	29	6564.265778			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.650404	7.163556	11.29120	157.6200



Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	3737.888741	415.320971	3.26	0.0158
Block	2	531.536889	265.768444	2.08	0.1534

---

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	6006.882139	546.080194	3.47	0.0095
Error	18	2833.337907	157.407662		
Corrected Total	29	8840.220046			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.679495	7.610561	12.54622	164.8528

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	5588.181343	620.909038	3.94	0.0064
Block	2	418.700796	209.350398	1.33	0.2893

---

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	9048.29593	822.57236	4.99	0.0014
Error	18	2967.68274	164.87126		
Corrected Total	29	12015.97867			

R-Square	Coeff Var	Root MSE	Wk_18 Mean
0.753022	7.537110	12.84022	170.3600

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	8300.035704	922.226189	5.59	0.0010
Block	2	748.260222	374.130111	2.27	0.1322

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**(b) Grafts plant height (cm) at Kapkatet site**

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The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

---

**Dependent Variable: Wk0**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2494.927877	226.811625	50.72	<.0001
Error	18	80.488116	4.471562		
Corrected Total	29	2575.415993			

R-Square	Coeff Var	Root MSE	Wk0 Mean
0.968748	8.189009	2.114607	25.82250

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2441.424836	271.269426	60.67	<.0001
Block	2	53.503042	26.751521	5.98	0.0102

---

**Dependent Variable: Wk2**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3213.895704	292.172337	28.77	<.0001
Error	18	182.767852	10.153770		
Corrected Total	29	3396.663556			

R-Square	Coeff Var	Root MSE	Wk2 Mean
0.946192	8.022402	3.186498	39.72000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	3116.089481	346.232165	34.10	<.0001

Block	2	97.806222	48.903111	4.82	0.0211
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**Dependent Variable: Wk 4**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	5745.182630	522.289330	15.74	<.0001
Error	18	597.114000	33.173000		
Corrected Total	29	6342.296630			

R-Square	Coeff Var	Root MSE	Wk_4 Mean
0.905852	8.858047	5.759601	65.02111

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	5452.435889	605.826210	18.26	<.0001
Block	2	292.746741	146.373370	4.41	0.0276

---

**Dependent Variable: Wk 6**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	5896.295778	536.026889	13.23	<.0001
Error	18	729.230296	40.512794		
Corrected Total	29	6625.526074			

R-Square	Coeff Var	Root MSE	Wk_6 Mean
0.889936	6.818470	6.364966	93.34889

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	9	5277.224593	586.358288	14.47	<.0001
Block	2	619.071185	309.535593	7.64	0.0040

---

**Dependent Variable: Wk 8**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	4273.846657	388.531514	7.20	0.0001
Error	18	971.738722	53.985485		
Corrected Total	29	5245.585380			

R-Square	Coeff Var	Root MSE	Wk_8 Mean
0.814751	6.117322	7.347482	120.1094

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	3708.451861	412.050207	7.63	0.0001
Block	2	565.394796	282.697398	5.24	0.0161

---

**Dependent Variable: Wk 10**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3460.692213	314.608383	7.37	0.0001
Error	18	768.310944	42.683941		
Corrected Total	29	4229.003157			

R-Square	Coeff Var	Root MSE	Wk_10 Mean
0.818323	5.117976	6.533295	127.6539

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2995.860750	332.873417	7.80	0.0001
Block	2	464.831463	232.415731	5.45	0.0141

---

**Dependent Variable: Wk 12**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	330.475111	302.770465	6.01	0.0004
Error	18	907.344519	50.408029		
Corrected Total	29	4237.819630			

R-Square	Coeff Var	Root MSE	Wk_12 Mean
0.785894	5.389798	7.099861	131.7278

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2827.491481	314.165720	6.23	0.0005
Block	2	502.983630	251.491815	4.99	0.0189

---

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3799.161315	345.378301	6.13	0.0004
Error	18	1014.609093	56.367172		
Corrected Total	29	4813.770407			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.789228	5.460577	7.507807	137.4911

Source	DF	SS	Mean Square	F Value	Pr > F
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Treatment	9	3317.982630	368.664737	6.54	0.0004
Block	2	481.178685	240.589343	4.27	0.0304

---

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	5818.738213	528.976201	10.96	<.0001
Error	18	868.362944	48.242386		
Corrected Total	29	6687.101157			

R-Square    Coeff Var    Root MSE    Wk\_16 Mean  
0.870144    4.763201    6.945674    145.8194

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	5299.167639	588.796404	12.20	<.0001
Block	2	519.570574	259.785287	5.39	0.0147

---

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	9246.38633	840.58058	8.27	<.0001
Error	18	1830.37607	101.68756		
Corrected Total	29	11076.76241			

R-Square    Coeff Var    Root MSE    Wk\_18 Mean  
0.834755    6.334408    10.08402    159.1944

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	8808.016481	978.668498	9.62	<.0001
Block	2	438.369852	219.184926	2.16	0.1448

---

**Appendix III -7: ANOVA table for Grafts plant growth rate (cm/ fortnight) at Chesingoro and Kapkatet sites**

**(a):Plant growth rate at Chesingoro site**

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 The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

-----  
**Dependent Variable: Wk 2**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1283.714176	116.701289	25.79	<.0001
Error	18	81.437204	4.524289		
Corrected Total	29	1365.151380			

R-Square	Coeff Var	Root MSE	Wk_2 Mean
0.940346	10.11297	2.127038	21.03278

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	1254.180824	139.353425	30.80	<.0001
Block	2	29.533352	14.766676	3.26	0.0617

-----  
**Dependent Variable: Wk 4**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	199.6782870	18.1525715	1.11	0.4089
Error	18	294.9619815	16.3867767		
Corrected Total	29	494.6402685			

R-Square	Coeff Var	Root MSE	Wk_4 Mean
0.403684	15.56314	4.048058	26.01056

Source	DF	SS	Mean Square	F Value	Pr > F
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Treatment	9	132.4274907	14.7141656	0.90	0.5465
Block	2	67.2507963	33.6253981	2.05	0.1575

---

**Dependent Variable: Wk 6**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	516.4351759	46.9486524	3.68	0.0071
Error	18	229.4490926	12.7471718		
Corrected Total	29	745.8842685			

R-Square	Coeff Var	Root MSE	Wk_6 Mean
0.692380	17.04797	3.570318	20.94278

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	508.7237130	56.5248570	4.43	0.0035
Block	2	7.7114630	3.8557315	0.30	0.7427

---

**Dependent Variable: Wk 8**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	192.8102500	17.5282045	0.90	0.5578
Error	18	350.3792778	19.4655154		
Corrected Total	29	543.1895278			

R-Square	Coeff Var	Root MSE	Wk_8 Mean
0.354959	18.34882	4.411974	24.04500

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	159.3478611	17.7053179	0.91	0.5380
Block	2	33.4623889	16.7311944	0.86	0.4400

---

**Dependent Variable: Wk 10**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	588.888917	53.535356	1.52	0.2068
Error	18	632.845574	35.158087		
Corrected Total	29	1221.734491			

R-Square	Coeff Var	Root MSE	Wk_10 Mean
0.482011	24.07345	5.929426	24.63056

Source	DF	SS	Mean Square	F Value	Pr > F
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Treatment	9	510.8857870	56.7650874	1.61	0.1849
Block	2	78.0031296	39.0015648	1.11	0.3513

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**Dependent Variable: Wk 12**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	656.2959907	59.6632719	3.67	0.0072
Error	18	292.4699815	16.2483323		
Corrected Total	29	948.7659722			

R-Square	Coeff Var	Root MSE	Wk_12 Mean
0.691736	46.64519	4.030922	8.641667

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	623.5206019	69.2800669	4.26	0.0043
Block	2	32.7753889	16.3876944	1.01	0.3844

---

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	194.0015833	17.6365076	2.57	0.0367
Error	18	123.6162407	6.8675689		
Corrected Total	29	317.6178241			

R-Square	Coeff Var	Root MSE	Wk_14 Mean
0.610802	53.69480	2.620605	4.880556

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	190.8235648	21.2026183	3.09	0.0200
Block	2	3.1780185	1.5890093	0.23	0.7958

---

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	447.7001389	40.7000126	3.87	0.0055
Error	18	189.4234630	10.5235257		
Corrected Total	29	637.1236019			

R-Square	Coeff Var	Root MSE	Wk_16 Mean
0.702690	44.85135	3.243998	7.232778

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	438.3371204	48.7041245	4.63	0.0028



Block	2	9.3630185	4.6815093	0.44	0.6478
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**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	374.8986944	34.0816995	2.86	0.0234
Error	18	214.6355741	11.9241986		
Corrected Total	29	589.5342685			

R-Square	Coeff Var	Root MSE	Wk_18 Mean
0.635923	62.70209	3.453143	5.507222

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	326.5900093	36.2877788	3.04	0.0213
Block	2	48.3086852	24.1543426	2.03	0.1609

---

**(b):Plant growth rate at Kapkatet site**

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The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

---

**Dependent Variable: Wk2**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	926.417211	84.219746	6.76	0.0002
Error	18	224.313227	12.461846		
Corrected Total	29	1150.730438			

R-Square	Coeff Var	Root MSE	Wk2 Mean
0.805069	25.40122	3.530134	13.89750

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	916.0826134	101.7869570	8.17	<.0001
Block	2	10.3345972	5.1672986	0.41	0.6667

---

**Dependent Variable: Wk 4**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	863.854185	78.532199	5.16	0.0011
Error	18	274.002444	15.222358		
Corrected Total	29	1137.856630			

R-Square	Coeff Var	Root MSE	Wk_4 Mean
0.759194	15.42060	3.901584	25.30111

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	774.6114444	86.0679383	5.65	0.0009
Block	2	89.2427407	44.6213704	2.93	0.0791

---

**Dependent Variable: Wk 6**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	286.5943333	26.0540303	1.08	0.4252
Error	18	433.1725185	24.0651399		
Corrected Total	29	719.7668519			

R-Square    Coeff Var    Root MSE    Wk\_6 Mean  
0.398177    17.31736    4.905623    28.32778

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	214.0987037	23.7887449	0.99	0.4820
Block	2	72.4956296	36.2478148	1.51	0.2484

---

**Dependent Variable: Wk 8**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	678.974472	61.724952	3.28	0.0125
Error	18	339.083574	18.837976		
Corrected Total	29	1018.058046			

R-Square    Coeff Var    Root MSE    Wk\_8 Mean  
0.666931    16.21892    4.340274    26.76056

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	677.4682315	75.2742479	4.00	0.0060
Block	2	1.5062407	0.7531204	0.04	0.9609

---

**Dependent Variable: Wk 10**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	201.5840185	18.3258199	2.00	0.0928
Error	18	165.0595000	9.1699722		
Corrected Total	29	366.6435185			

R-Square    Coeff Var    Root MSE    Wk\_10 Mean  
0.549809    40.13809    3.028196    7.544444

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	184.3272222	20.4808025	2.23	0.0702
Block	2	17.2567963	8.6283981	0.94	0.4086

---

**Dependent Variable: Wk 12**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	76.2374352	6.9306759	1.52	0.2072
Error	18	81.9896111	4.5549784		
Corrected Total	29	158.2270463			

R-Square    Coeff Var    Root MSE    Wk\_12 Mean  
0.481823    52.38826    2.134240    4.073889

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	66.96297222	7.44033025	1.63	0.1794
Block	2	9.27446296	4.63723148	1.02	0.3812

---

**Dependent Variable: Wk 14**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	139.2130556	12.6557323	2.58	0.0359
Error	18	88.2549444	4.9030525		
Corrected Total	29	227.4680000			

R-Square    Coeff Var    Root MSE    Wk\_14 Mean  
0.612012    38.42019    2.214284    5.763333

Source	DF	Type III SS	Mean Square	F Value	Pr > F
Treatment	9	118.3230000	13.1470000	2.68	0.0358
Block	2	20.8900556	10.4450278	2.13	0.1478

---

**Dependent Variable: Wk 16**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	429.3416944	39.0310631	6.55	0.0003

Error	18		107.3100556	5.9616698		
Corrected Total	29		536.6517500			
	R-Square	Coeff Var	Root MSE	Wk_16 Mean		
	0.800038	29.31743	2.441653	8.328333		
Source	DF	SS	Mean Square	F Value	Pr > F	
Treatment	9	410.8656389	45.6517377	7.66	0.0001	
Block	2	18.4760556	9.2380278	1.55	0.2394	

---

**Dependent Variable: Wk 18**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	639.663880	58.151262	2.11	0.0766
Error	18	495.215426	27.511968		
Corrected Total	29	1134.879306			

	R-Square	Coeff Var	Root MSE	Wk_18 Mean
	0.563640	39.21634	5.245185	13.37500

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	604.2794907	67.1421656	2.44	0.0512
Block	2	35.3843889	17.6921944	0.64	0.5373

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**Appendix III-8: ANOVA table for yield parameters at Chesingoro and Kapkatet sites**

**(a):Yield parameters for Chesingoro site**

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 The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

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**Dependent Variable: Days to first harvest**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	491.7000000	44.7000000	4.74	0.0018
Error	18	169.8000000	9.4333333		
Corrected Total	29	661.5000000			

R-Square	Coeff Var	Root MSE	Days to first harvest Mean
0.743311	5.341519	3.071373	57.50000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	457.5000000	50.8333333	5.39	0.0012
Block	2	34.2000000	17.1000000	1.81	0.1918

-----  
**Dependent Variable: No of fruits picked**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	94.7066296	8.6096936	1.43	0.2409
Error	18	108.2230370	6.0123909		
Corrected Total	29	202.9296667			

R-Square	Coeff Var	Root MSE	No of fruits picked Mean
0.466697	16.79848	2.452018	14.59667

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	92.79707407	10.31078601	1.71	0.1578
Block	2	1.90955556	0.95477778	0.16	0.8543

-----

**Dependent Variable: Yield of picked fruits, g/plant**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	831911.756	75628.341	3.02	0.0183
Error	18	451077.001	25059.833		
Corrected Total	29	1282988.758			

R-Square	Coeff Var	Root MSE	Yield of picked fruits g/plant Mean
0.648417	20.69550	158.3030	764.9150

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	816467.2174	90718.5797	3.62	0.0097
Block	2	15444.5391	7722.2695	0.31	0.7386

-----

**Dependent Variable: Fruit wt. (g)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	2551.030815	231.911892	3.45	0.0097
Error	18	1208.760847	67.153380		
Corrected Total	29	3759.791662			

R-Square	Coeff Var	Root MSE	Fruit wt. (g) Mean
0.678503	15.62582	8.194717	52.44342

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2351.694738	261.299415	3.89	0.0068
Block	2	199.336077	99.668038	1.48	0.2531

-----

**Dependent Variable: No. of fruits set / plant**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	146.0781111	13.2798283	1.82	0.1259
Error	18	131.5922963	7.3106831		
Corrected Total	29	277.6704074			

R-Square	Coeff Var	Root MSE	No of fruits set/ plant Mean
0.526085	17.39790	2.703827	15.54111

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	140.9489259	15.6609918	2.14	0.0807

Block	2	5.1291852	2.5645926	0.35	0.7088
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**Dependent Variable: No. of clusters**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	20.36296296	1.85117845	3.74	0.0065
Error	18	8.911111111	0.49506173		
Corrected Total	29	29.27407407			

R-Square	Coeff Var	Root MSE	No. of clusters Mean
0.695597	13.08359	0.703606	5.377778

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	16.75555556	1.86172840	3.76	0.0081
Block	2	3.60740741	1.80370370	3.64	0.0469

---

**Dependent Variable: No. of fruits set/ cluster**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	3.60722901	0.32792991	0.96	0.5105
Error	18	6.13631768	0.34090654		
Corrected Total	29	9.74354669			

R-Square	Coeff Var	Root MSE	No. of fruits set/cluster Mean
0.370217	19.87045	0.583872	2.938394

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	2.67036767	0.29670752	0.87	0.5672
Block	2	0.93686134	0.46843067	1.37	0.2784

---



**(b): Yield parameters for Kapkatet site**

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The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

---

**Dependent Variable: Days to first harvest**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	298.5000000	27.1363636	2.10	0.0779
Error	18	232.2000000	12.9000000		
Corrected Total	29	530.7000000			

R-Square	Coeff Var	Root MSE	Days to first harvest Mean
0.562465	5.517138	3.591657	65.10000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	236.7000000	26.3000000	2.04	0.0948
Block	2	61.8000000	30.9000000	2.40	0.1196

---

**Dependent Variable: Yield of picked fruits (g/plant)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	207552.3433	18868.3948	7.15	0.0001
Error	18	47504.8579	2639.1588		
Corrected Total	29	255057.2012			

R-Square	Coeff Var	Root MSE	Yield of picked fruits (g/plant) Mean
0.813748	13.21023	51.37274	388.8861

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	199441.4650	22160.1628	8.40	<.0001
Block	2	8110.8782	4055.4391	1.54	0.2420

-----  
**Dependent Variable: No. of fruits picked/ plant**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	480.5069259	43.6824478	8.28	<.0001
Error	18	94.9119259	5.2728848		
Corrected Total	29	575.4188519			

R-Square	Coeff Var	Root MSE	No of fruits picked/plant Mean
0.835056	17.50062	2.296276	13.12111

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	475.3610741	52.8178971	10.02	<.0001
Block	2	5.1458519	2.5729259	0.49	0.6218

-----  
**Dependent Variable: Fruit wt. (g)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	644.3119422	58.5738129	5.75	0.0006
Error	18	183.2716081	10.1817560		
Corrected Total	29	827.5835503			

R-Square	Coeff Var	Root MSE	Fruit wt. (g) Mean
0.778546	10.34954	3.190886	30.83120

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	641.0812944	71.2312549	7.00	0.0002
Block	2	3.2306478	1.6153239	0.16	0.8545

-----  
**Dependent Variable: Total no. of fruits set**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	786.2660000	71.4787273	8.22	<.0001
Error	18	156.4837778	8.6935432		
Corrected Total	29	942.7497778			

R-Square	Coeff Var	Root MSE	Total no. of fruits set Mean
0.834013	18.47419	2.948482	15.96000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	776.9364444	86.3262716	9.93	<.0001
Block	2	9.3295556	4.6647778	0.54	0.5938

---

**Dependent Variable: No. of clusters**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	25.46666667	2.31515152	4.39	0.0028
Error	18	9.49629630	0.52757202		
Corrected Total	29	34.96296296			

R-Square	Coeff Var	Root MSE	No of clusters Mean
0.728390	12.57130	0.726342	5.777778

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	24.37037037	2.70781893	5.13	0.0016
Block	2	1.09629630	0.54814815	1.04	0.3741

---

**Dependent Variable: No of fruits set per cluster**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	9.26376025	0.84216002	3.77	0.0063
Error	18	4.02222877	0.22345715		
Corrected Total	29	13.28598902			

R-Square	Coeff Var	Root MSE	No of fruits set per cluster Mean
0.697258	17.34554	0.472713	2.725269

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	9.24770494	1.02752277	4.60	0.0029
Block	2	0.01605531	0.00802765	0.04	0.9648

---

**Appendix III-9: ANOVA table for Fruit quality at Chesingoro and Kapkatet sites**

**(a): Fruit quality parameters at Chesingoro site**

The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used	30	

**Dependent Variable: Fruit diameter (cm)**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	1.51176979	0.13743362	7.73	<.0001
Error	18	0.31996437	0.01777580		
Corrected Total	29	1.83173416			

R-Square	Coeff Var	Root MSE	Fruit_diameter_cm Mean
0.825322	3.244869	0.133326	4.108823

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	1.42349550	0.15816617	8.90	<.0001
Block	2	0.08827429	0.04413715	2.48	0.1116

**Dependent Variable: pH**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.31837000	0.02894273	2.89	0.0223
Error	18	0.18044667	0.01002481		
Corrected Total	29	0.49881667			

R-Square	Coeff Var	Root MSE	PH Mean
0.638251	2.199722	0.100124	4.551667

Source	DF	SS	Mean Square	F Value	Pr > F
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Treatment	9	0.31368333	0.03485370	3.48	0.0117
Block	2	0.00468667	0.00234333	0.23	0.7939

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**Dependent Variable: BRIX**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	5.05785667	45980515	8.45	<.0001
Error	18	0.97924000	0.05440222		
Corrected Total	29	6.03709667			

R-Square	Coeff Var	Root MSE	BRIX Mean
0.837796	3.844873	0.233243	6.066333

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	5.04423000	0.56047000	10.30	<.0001
Block	2	0.01362667	0.00681333	0.13	0.8830

---

**(b): Fruit quality parameters for Kapkatet site**

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The GLM Procedure

Class Level Information

Class	Levels	Values
Treatment	10	9 10 11 12 13 14 15 16 17 18
Block	3	1 2 3
Number of Observations Used		30

---

**Dependent Variable: Fruit diameter, cm**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.65904223	0.05991293	3.46	0.0097
Error	18	0.31195220	0.01733068		
Corrected Total	29	0.97099442			

R-Square	Coeff Var	Root MSE	Fruit_diameter_cm Mean
0.678729	3.611834	0.131646	3.644852

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	0.65420499	0.07268944	4.19	0.0047
Block	2	0.00483724	0.00241862	0.14	0.8707

---

**Dependent Variable: PH**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	0.38552667	0.03504788	2.82	0.0246
Error	18	0.22339333	0.01241074		
Corrected Total	29	0.60892000			

R-Square	Coeff Var	Root MSE	PH Mean
0.633132	2.445204	0.111404	4.556000

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	0.36618667	0.04068741	3.28	0.0153
Block	2	0.01934000	0.00967000	0.78	0.4737

---

**Dependent Variable: BRIX**

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	11	12.79370667	1.16306424	4.30	0.0031
Error	18	4.86368000	0.27020444		
Corrected Total	29	17.65738667			

R-Square	Coeff Var	Root MSE	BRIX Mean
0.724553	7.697876	0.519812	6.752667

Source	DF	SS	Mean Square	F Value	Pr > F
Treatment	9	11.72072000	1.30230222	4.82	0.0022
Block	2	1.07298667	0.53649333	1.99	0.1663

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