

**Evaluation of Grafting Rootstocks on Plant Growth, Fruit Yield and Quality in
Tomato (*Lycopersicon esculentum* Mill).**

By

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Abstract

Grafting is the process of combining two parts of plants to form a single plant. Grafting is a common technique in trees and vine crops and is becoming popular in annual vegetable production in order to control biotic and abiotic stresses, improve fruit yield and quality. The objectives of the study were to determine the (a) compatibility of the tomato scion x rootstocks combinations (b) effect of rootstocks on vegetative growth parameters (c) effect of tomato rootstocks on fruit quality and yield of tomato. The experiments were conducted under a protected environment (in a high tunnel) at the University of Venda, Thohoyandou, South Africa. Four tomato rootstocks ('Powapak', 'Everest', 'Matterhorn' and 'Golf') and two scion cultivars ('Money Maker' and 'Rodade') were used to develop 10 scion x rootstock combinations. The tube grafting method was used. Seedlings were transplanted in the polyethylene bags inside the high tunnel. Growth parameters, including plant height, stem diameter, number of leaves per cluster as well as yield attributes were measured. In addition, the chemical fruit quality parameters including the total soluble solids and pH were determined. A randomized complete block design with four replications was used for the study. Quantitative data sets of the variables were subjected to analysis of variance. There was a high plant survival rate and the number of clusters per plant showed marked improvement in some grafting combinations particularly in 'Rodade x Everest' and 'Money Maker x Everest'. A few individual stunted plants were observed in the tunnel. A significant seasonal effect was observed on some of the growth and productivity attributes. The pH showed a narrow range of values and there was significant interaction between sampling time and genotype. The results demonstrated the viability of producing grafted tomatoes under tunnel conditions.

Key words: grafting, rootstock, scion, tomato, yield.

Declaration

I, Mothapo Maurine Ramokone, hereby declare that the research submitted for the degree of Master of Science in Agriculture at University of Venda is my own original work and has not been submitted previously to this or any other university. I further declare, that the sources cited or quoted herein have been dully acknowledged by means of a complete list of references.

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As supervisors, we agree to the submission of this dissertation

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Co-supervisor: Prof. ET Gwata

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Dedication

I dedicate this dissertation to my mother, Meriam Rakgadi Mothapo and to my son Itumeleng Mankwe Mothapo.

Table of Contents

Abstract.....	ii
Declaration.....	iii
Acknowledgements.....	iv
Dedication.....	v
List of Figures.....	viii
List of Tables.....	x
List of Appendices.....	xi
List of Abbreviations.....	xii
1.0 CHAPTER ONE: GENERAL INTRODUCTION.....	1
1.1 Introduction.....	1
1.2 Problem statement.....	2
1.3 Justification.....	2
1.4 Objectives of the study.....	3
1.5 Hypotheses.....	3
1.6 Dissertation outline.....	3
2.0 CHAPTER TWO: LITERATURE REVIEW.....	4
1.1 Introduction.....	4
2.2 Grafting tomatoes.....	4
2.3 Cultivation of grafted crop plants.....	5
2.4 Effect of grafting on growth and productivity parameters of tomato.....	5
2.5 Effect of grafting on quality parameters.....	5
2.6 Benefits of grating.....	6
2.7 Disadvantages of grafting.....	6
2.8 Major areas of tomato production in South Africa.....	7
2.9 Quality attributes of tomato.....	7

3.0 CHAPTER THREE: MATERIALS AND METHOD.....	9
3.1 Site selection.....	9
3.2 Tomato cultivars.....	9
3.3 Grafting tomato plants.....	10
3.4 Trial management.....	12
3.5 Measurements.....	13
3.5.1 Compatibility of tomato scion and rootstock combinations.....	13
3.5.2 Tomato growth parameters at the vegetative stage.....	13
3.5.3 Tomato fruit quality and yield.....	14
3.6 Experimental design and statistical analysis.....	14
4.0 CHAPTER FOUR: RESULTS.....	15
4.1 Grafting compatibility.....	15
4.2 Impact of grafting on growth parameters.....	16
4.3 Impact of grafting on fruit productivity and quality parameters.....	28
4.4 Correlation between growth production and quality traits.....	28
5.0 CHAPTER FIVE: DISCUSSION.....	33
6.0 CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS.....	36
REFERENCES.....	38
APPENDICES.....	45

List of Figures

Fig. 3.1 The floor of a standard plastic tunnel covered with a black plastic sheeting in order to raise the temperature inside the tunnel.....	9
Fig. 3.2 Grafting tube was inserted onto the rootstock (a) in order enable insertion of the scion (b) onto the rootstock.....	11
Fig.3.3 The healing chamber made of polyvinyl chloride which was used in the study....	12
Fig. 4.1 The success rate of grafting various tomato scion x rootstock combinations.....	15
Fig. 4.2 The graft union in tomato indicating (a) normal compatibility between Rodade x Rodade' and (b) delayed (partial) compatibility between 'Rodade x Powapak'.....	16
Fig. 4.3 Individual plants were stunted and possessed crinkled leaves suggesting viral infection in some of the tomato scion x rootstock combinations that were evaluated in a tunnel at Thohoyandou (Limpopo Province).....	20
Fig. 4.4 Seasonal variation in plant height among tomato scion x rootstock combinations that were evaluated in a tunnel during 2016 (red bars) and 2017 (green bars) at Thohoyandou (Limpopo Province).....	21
Fig. 4.5 Some of the tomato scion x rootstock combinations were markedly tall, reaching	

the roof of the tunnel at Thohoyandou (Limpopo Province).....	22
Fig. 4.6 Seasonal variation in the number of leaves per cluster among tomato scion x rootstock combinations that were evaluated in a tunnel during 2016 (red bars) and 2017 (green bars) at Thohoyandou (Limpopo Province).....	25
Fig. 4.7 The magnitude of increment in three growth parameters among tomato cultivars that were grafted on a ‘Rodade’ rootstock and subsequently evaluated in the tunnel at Thohoyandou (Limpopo Province).	26
Fig. 4.8 The magnitude of increment in three growth parameters among tomato cultivars that were grafted on a ‘Money Maker’ rootstock and subsequently evaluated in the tunnel at Thohoyandou (Limpopo Province).	26
Fig. 4.9 Variation in fruit load, fruit size, time to ripening within individual plants among grafted tomato plants that were evaluated in a tunnel at Thohoyandou (Limpopo Province)	29
Fig. 4.10 Variation in total fruit fresh weight among grafted tomato plants that were evaluated in a tunnel at Thohoyandou (Limpopo Province).	30
Fig. 4.11 The relationship between fruit number per plant and the number of leaves per cluster among various scion x rootstock combinations of tomato cultivars that were evaluated under tunnel conditions at Thohoyandou	32

List of Tables

Table 1.1 The major tomato production areas in South Africa.....	7
Table 3.1. Tomato cultivars and the scion x rootstock grafting combination that were used in the in the study.....	10
Table 4.1 Analysis of variance for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel at Thohoyandou (Limpopo Province).....	18
Table 4.2 Means for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel during 2016 at Thohoyandou (Limpopo Province).....	19
Table 4.3 Combined analysis of variance for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel for two seasons at Thohoyandou (Limpopo Province).....	23
Table 4.4 Means from the combined analysis of variance for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel for two seasons at Thohoyandou (Limpopo Province).....	27
Table 4.5 Pearson correlation coefficients among quantitative traits of grafted tomato plants that were evaluated in a tunnel at Thohoyandou (Limpopo Province).....	31

List of Appendices

Appendix I Composition of a 25.0 kg bag of fertilizer that was utilized for raising grafted tomato plants in the tunnel.....	40
Appendix II Crop inspection in the tunnel was conducted regularly.....	46
Appendix III Means for growth parameters of tomato scion x rootstock combination that were evaluated in the tunnel during 2017 at Thohoyandou (Limpopo Province).....	47

List of Abbreviations

ANOVA	- analysis of variance
DAFF	- Department of Agriculture Forest and Fisheries
FD	- fruit diameter
FY	- total fruit yield per plant
FW	- fresh weight per plant
NB	- number of branches
NLC	- number of leaves per cluster
NWS	- number of wilted scions
PHT	- plant height
PVC	- polyvinyl chloride
RCBD	- randomized complete block design
SD	- stem diameter
SR	- survival rate
TSS	- total soluble solutes

1.0 CHAPTER ONE: GENERAL INTRODUCTION

1.1 Introduction

Tomato (*Lycopersicon esculentum* Mill), is a member of Solanaceae family. It originated in tropical America i.e. the Andean region which includes Peru, Ecuador, Bolivia, Chile and Colombia (Salunkhe *et al.*, 1987). The tomato has adapted a wide range of climatic conditions including tropical, subtropical and temperate conditions.

Botanically, it is a dicotyledonous herbaceous plant with chromosome number $2n=24$ (Jenkins, 1948). It grows up to 3.0m tall. The leaves are hairy, arranged spirally and also vary in size. In addition, it bears flowers which have six yellow petals and six stamens that unite in the area of anthers to form a cone around the female organ yellow flowers (Heuvelink, 2005). Tomato flowers are self-fertile possessing both male and female parts. Fruits differ in size and are usually round to lobed, fleshy; smooth skinned and can be red, pink, purple, brown, orange or yellow in colour when ripe. Flat, slightly curved, hairy, light brown seeds are produced (Heuvelink, 2005).

Some tomato cultivars have an erect or prostrate growth habits wherein tall varieties are called indeterminate which keep on growing after flowering. They are the best choice for long harvest time and they are normally staked or trellised. Other cultivars are determinate types, which stop growing after flowering and do not need staking. The plant can reach up to 2.0m tall or more particularly in a controlled environment such as a tunnel. The initial harvest of tomato is done around 45-55 days after flowering or 90-120 days after sowing. The same plant can be harvested for more than two years continuously. In general tomato cultivars differ in shape, colour and also size. The colours range from yellow to red (Naika *et al.*, 2005).

Tomato is rich in valuable nutritional components with antioxidants and a mixture of different carotenoids, including vitamin A (Vinkovic-Vrcek *et al.*, 2011) and contains a wide range of vitamins including riboflavin (Lester, 2006). Lycopene, one of nature's most powerful antioxidant, is present in tomatoes and especially when tomatoes are cooked. This has been found beneficial in preventing prostate cancer. The consumption of tomatoes rich in lycopene leads directly to a decreased incidence of cancer in mouth, pharynx, oesophagus, stomach, large intestine and

rectum. Americans use the fresh fruit orally for kidney and liver problems, as a cathartic and to keep good digestion.

In South Africa, tomato is a major vegetable crop commonly grown by both poor and rich farmers. It is used as a fresh vegetable in South Africa or as a sauce for flavour enhancement. Currently, it is one of the main vegetables used for hawking by small-scale entrepreneurs in the informal sector. The crop is also grown commercially. The area under cultivation both as a field crop and in tunnels is increasing (DAFF, 2011).

Tomato can be grown in most soil types. Sandy soil and loamy soil with adequate supply of organic matter, good moisture holding and drainage capacity are ideal for tomato cultivation. The optimum temperatures for seed germination, vegetative growth and reproductive growth are 20°C, 25°C, 18-22°C respectively. Tomato requires high light intensity. The optimum relative humidity of 60-70% is ideal for tomato production. About 9-11 hours' day length is required for flowering.

1.2 Problem statement

Grafting of tomato on selected rootstocks has been used in tomato production in order to provide abiotic and biotic stress tolerance, enhance nutrients uptake as well as increase yield and quality. However, there are contradictory reports on fruit quality enhancement. In addition, the effect of grafting on the production and quality of tomato have not been determined adequately. In particular, the influence of rootstock on plant growth, fruit yield and quality of tomato germplasm which is used in the Limpopo Province of South Africa has not been determined sufficiently. This limits the selection of tomato scion x rootstock combinations that produce optimum growth and productivity for growers.

1.3 Justification of the study

Tomato is an important crop in South Africa. Grafting provides a rapid method for improving growth and productivity of tomato. However, the effect of grafting various scion x rootstock combinations of tomato have not been determined sufficiently in the Limpopo Province. Such information will benefit tomato improvement programs and tomato end-users in the region.

1.4 Objectives of the study

The overall objective of the study was to evaluate the scion x rootstock performance of grafted tomato. The specific objectives of the study were to:

- (i) evaluate compatibility between tomato scion x rootstock combinations
- (ii) determine the influence of tomato scion x rootstock combinations on vegetative growth parameters
- (iii) determine the influence of scion x rootstock combinations on tomato fruit quality and yield.

1.5 Hypotheses of the study

The study tested the following null hypotheses:

- (i) there were no significant differences in compatibility between tomato scion x rootstock combinations
- (ii) there were no significant differences in vegetative growth parameters among tomato scion x rootstock combinations
- (iii) there were no significant differences in fruit quality and yield between the tomato scion x rootstock combinations.

1.6 Dissertation outline

The dissertation is divided into six chapters. The first chapter introduces the tomato crop and defines the problem statement as well as the study objectives. In chapter two, relevant aspects of grafting in tomato are reviewed. In chapter three, the materials as well as the methods that were used in the study are described and followed by a presentation of the results of the study in chapter four. In chapter five, the results are discussed. In chapter six, the findings from the study are summarized. In the final component of the document, a combined list of references for all the chapters is followed by a list of appendices.

2.0 CHAPTER TWO: LITERATURE REVIEW

2.1 Introduction

Across South Africa, tomato is a source of income and food security for small holder farmers. Limpopo Province is the main producing area in South Africa with a total annual production of approximately 227 990.0t (DAFF, 2011). Tomato is a high-value vegetable crop that is widely consumed fresh or processed. It is cultivated on about 6000 ha and contributes 24% of the total vegetable production in South Africa. The commercial sector contributes 95.0 % of the total produce while the smallholder sector contributes only 5.0 % (DAFF, 2011). The demand for tomato is high in South Africa. Therefore, high yielding cultivars that produce good quality tomato are desirable for growers in order to meet the demand by end-users.

2.2 Grafting tomatoes

Apart from conventional breeding methods, the fruit yield of tomato can be improved through the use of techniques such as grafting and tissue culture. In comparison, grafting is more widely used partly because it is relatively easy to apply and cheaper than tissue culture. By definition, grafting is the art of joining two pieces of living plant tissue so that they will unite and grow as a composite plant. The scion is the top part of the plant whose fruit one desires and the rootstock is the lower portion of the composite plant which develops into the root system.

The technique of grafting was used successfully in tomato cultivation and fruit trees such as peaches, almond, and pears. In the technique, the rootstock is utilized to minimize abiotic and biotic stresses (Yassin and Hassen, 2015) as well as improve the yield and quality of fruits. The interaction between scion x rootstock leads to vigorous root system development and higher absorption of water and mineral resulting in enhancement of fruit yield and quality.

The use of this technique was expanded to include the improvement of thermal tolerance in plants. In addition, intensification of vegetable crop production contributed to the increase in the incidence of soil-born pests and pathogens. However, the impact of the pests and pathogens can be mitigated by vegetable grafting particularly in organic production where the use of synthetic pesticides is discouraged. Ultimately all these approaches positively influence the fruit yield and quality in the various crops.

2.3 Cultivation of grafted crop plants

Grafted vegetables were planted in both tunnel and greenhouse environmental conditions (Khah *et al.*, 2006). This allowed farmers to move away from traditional crop rotation. In Morocco, grafting is used commercially in green house tomato, melon and watermelon crops to manage root-not nematodes and other soil borne diseases (Albedalhag, 2004; Besri, 2001). However, grafted fruits trees are planted in the open field since they require more space than that available under tunnel or greenhouse conditions. Nonetheless, the freshly grafted seedlings of fruit trees may require acclimation in controlled environments or to transplant in the open field (khah *et al.*, 2006).

2.4 Effect of grafting on growth and productivity parameters of tomato

Lee (1994) reported that grafted plants were taller and more vigorous than ungrafted plants in tomato production. The improved productivity of grafted plants was attributed to the vigor of the rootstock and scion x rootstock interaction, which gives positive influence on plant nutrients and water absorption (Anoli *et al.*, 2010).

Pongonyi *et al.*, (2005) examined the effect of grafting on the yield and fruit characteristics of tomato cultivars in which ‘Lemance F1’ was used as a scion with ‘Beaufort’ as rootstock and reported higher yield from the grafted plants than those which were not grafted. In the same study, soluble solids and sugars in the fruit were all higher in plants grafted on scarlet eggplant than those on tomato rootstock.

2.5 Effect of grafting on quality parameters

In a study designed to understand the effect of grafting on the quality of tomato, a lower Brix value was reported for grafted tomato (Romano and Paratore, 2001). In addition, soluble solids and sugars were higher in plants that were grafted on scarlet eggplant than those on tomato rootstock (Pongonyi *et al.*, 2005). Grafting appears to have effect on tomato quality although the results obtained so far have largely been inconsistent. Although results are not conclusive, tomato fruit quality does not seem to be affected positively or negatively by grafting (Khah *et al.*, 2006;

Romano and Paratore, 2001). Increasing consumer demand for high quality vegetable crops makes it essential to carefully select rootstock x scion combination that can ensure high quality.

2.6 Benefits of grafting

Grafting has been useful in fruits and vegetable crops for different purposes, such as disease resistance, tolerance to excessive temperature, salinity and drought, enhanced water and nutrient uptake and increased plant vigor (Jung-Myung, 1994). Grafting is one of the most popular techniques that was used in vegetables production particularly for managing soil-borne diseases and nematodes (Louws *et al.*, 2010). Another important benefit of grafting is protection against abiotic stresses such as high/low temperature and salinity (Colla *et al.*, 2010; San-Chez-Rodriguez *et al.*, 2014). In addition, grafting decreases the need for chemical or fertilizer application (Edelstein, 2004). Grafting can also be useful in tolerating drought or excessive moisture (Schwarz *et al.*, 2010). Furthermore, grafting can provide a strong trunks for some ornamental shrubs and trees. In these cases, a graft is made at a desired height on a stock plant with a strong stem. Grafting influences absorption and translocation of nutrients (Ruiz *et al.*, 1997). It has been suggested that these conditions allow grafted plants to produce higher yields, sometimes with improved fruit yield (Xu *et al.*, 2006 and Zhu *et al.*, 2008).

Grafting can also accelerate the maturity of hybrids in fruit tree breeding programs. Hybrid seedlings may take ten or more years to flower on their own roots hence grafting can reduce the time to flowering and shorten the breeding program.

2.7 Disadvantages of grafting

Even though there are several exciting benefits for grafting, some drawbacks have discouraged this technology from use. For instance, rootstock x scion incompatibility which encourages undergrowth or overgrowth of the scion may occur, leading to decrease in water and nutrient flow through the grafted union. This will cause the plant to wilt (Lee and Oda, 2003).

According to Romano and Paratore (2001), grafting does not improve yield when selection of the rootstock is not suitable. Sex expression and flowering order are controlled by plant hormones hence the scion x rootstock combination may alter the quantity of hormones produced (Sato,

1996). The rootstock can also affect flowering time while the scion variety can affect final fruit size, yield and quality (Davis *et al.*, 2008a). Grafting requires a significant amount of labour and time resulting in high cost for the grafted seedlings. The equipment for grafting is also expensive (Kubota *et al.*, 2008).

2.8 Major areas of tomato production in South Africa

Tomato is cultivated in the entire country mostly in summer season. However, three provinces constitute the major tomato production areas (Table 1.1). During winter, the production is quite low but the crop can be produced particularly in areas where there is no frost or under protected structures such as greenhouses or tunnels.

Table 1.1 The major tomato production areas in South Africa (DAFF,2011)

Province	Area (ha)	Quantity (t)
Limpopo	3590.0	87290.0
Mpumalanga	770.0	36500.0
Eastern Cape	450.0	36000.0

2.9 Quality attributes of tomato

Quality is defined as a degree of excellence, a high standard and value. Tomato fruit quality for fresh consumption is determined by appearance (size, shape, colour and absence of defects and decay). Kramer (1965) stated that the appearance of the product determined whether a product is accepted or rejected. Therefore appearance is one of the most important and critical quality attribute. Texture is also an important quality for consideration in the tomato fruit. It is affected by traits such as cellular organelle and biochemical constitutes, water content and cell wall composition (Sams, 1999). Therefore, any external factor affecting these traits can change texture and can also lead to changes in product quality. Firmness is also one of the typical attributes used to describe the texture. In addition, flavour (i.e. a composite of sensory responses in the nose and mouth to aroma and taste), is composed of acids, sugar and volatiles.

3.0 CHAPTER THREE: MATERIALS AND METHODS

3.1 Site selection

The study was carried out for two consecutive seasons (2015-2016) in a standard plastic tunnel (10.0 x 30.0 m) at the University of Venda Experimental Farm, Thohoyandou (22°35' 14.0 S; 30° 15' 50.3 E) at an altitude of 595m above sea level. The tunnel was positioned north-south with excellent light cover during the day, in Limpopo Province (South Africa). The area is characterized by high temperatures (30-45 °C) during the summer season and low temperatures (10-20 °C) in winter. The floor of the tunnel was covered with a black plastic sheet (Fig. 3.1) which helped raise the temperature in the tunnel and control weeds.



Fig. 3.1 The floor of a standard plastic tunnel covered with a black plastic sheeting in order to raise the temperature inside the tunnel

3.2 Tomato cultivars

Six tomato cultivars were used in the study to develop 10 scion x rootstock grafting combinations (Table 3.1). The scion cultivars were mainly popular commercial types that are cultivated by

growers in Limpopo Province. The seed of the scion cultivars was purchased from retail markets in Thohoyandou (Limpopo Province) and the seeds of the rootstock cultivars were purchased from Juvon Agricultural Services (Thohoyandou, Limpopo). Two controls were constituted from grafting 'Rodade' x 'Rodade' and 'Money Maker' x 'Money Maker' (Table 3.1). The commercial tomato cultivars 'Money Maker' and 'Rodade' were used as scions whereas 'Powapak', 'Everest', 'Matterhorn' and 'Golf' were used as rootstocks. All the cultivars were selected randomly.

3.1 Tomato cultivars and the scion x rootstock grafting combination that were used in the in the study.

Cultivars	Scion X Rootstock Combination
1. 'Everest' (E)	1. 'Rodade' x 'Powapak'
2. 'Golf' (G)	2. 'Rodade' x 'Everest'
3. 'Matterhorn' (M)	3. 'Rodade' x 'Matterhorn'
4. 'Money Maker' (MM)	4. 'Rodade' x 'Golf'
5. 'Powapak' (P)	5. 'Rodade' x 'Rodade' (control)
6. 'Rodade' (R)	6. 'Money Maker' x 'Powapak'
	7. 'Money Maker' x 'Everest'
	8. 'Money Maker' x 'Matterhorn'
	9. 'Money Maker' x 'Golf'
	10. 'Money Maker' x 'Money Maker' (control)

3.3 Grating of tomato plants

The seeds were sown inside seedling flat trays with hygromix growing media in the controlled environment in the green house. Seedlings were watered as necessary using the feel and appearance method to test the requirement for irrigation. Seeds of the scion cultivars were sown 5 days earlier than seeds of the rootstocks in order to ensure the similar stem diameter at the grafting time because of the differences in plant vigor.

The seedlings were grafted by hand, applying the tube grafting method (Oda, 1995; Rivard and Louws, 2008) when the scion had developed at least two real leaves and the rootstock had developed at least three real leaves (Fig.3.2). Both the rootstock and scion were cut at 45° to the

vertical axis of the stem. The scions were aligned with the rootstock to ensure the contact of vascular tissue between the two plants. The grafting procedure used silicone clip (2.0 mm) cut to 1.0 cm length and slit down one side. The grafted plants were placed in 4.1 x 1.4 m healing chamber (Fig. 3.3) constructed from polyvinyl chloride (PVC) frame covered by clear plastic, overlaid by one layer of polyethylene black plastic to maintain humidity (90.0 – 95.0%). The polyethylene plastic was used during the first week to minimize water loss of grafted seedlings through transpiration. Within the chamber, irrigation was done through mist spraying. Temperature was set at 22°C with no artificial lighting during the healing period. Seven days after grafting, the black plastic was removed gradually and the healing chamber was opened to increase light and lower the humidity. Fourteen days after grafting, the seedlings were taken out to the shade net for hardening off.

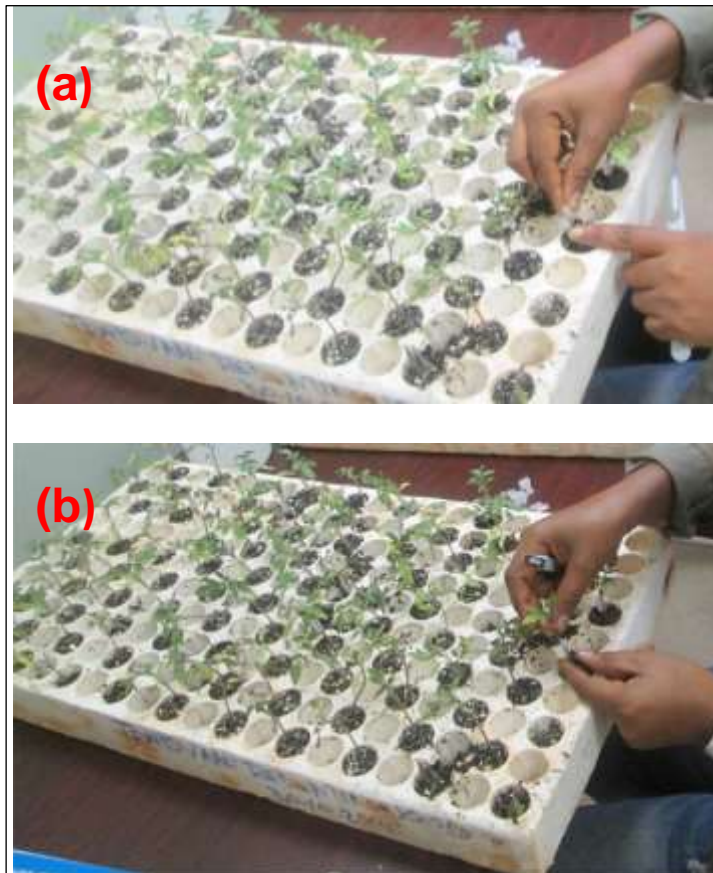


Fig. 3.2 Grafting tube was inserted onto the rootstock (a) in order enable insertion of the scion (b) onto the rootstock.



Fig.3.3 The healing chamber made of polyvinyl chloride which was used in the study.

3.4 Trial management

Seven days after hardening off, the plants were transplanted in polyethylene bags (7.0 L) in the tunnel structure. Immediately after transplanting, temperature readings in the tunnel were recorded in the morning and afternoon using a thermometer. Water soluble fertilizer (Haifa Cal) and Haifa Solution Hydroponic Mix were used for nutrification / fertigation. For the plants, 2.0 kg Haifa Cal fertilizer was diluted with 5000.0 L water. In addition, 1.0 kg Haifa Solution Hydroponic Mix (Appendix I) was also diluted with 5000.0 L water. This fertilizer was applied at the initial growing (seedling) stage.

At flowering initiation, 4.0 kg Haifa Cal (Appendix I) was added to the fertilizer for the seedlings in order to constitute the fertilizer that was subsequently used for adult plants until harvesting stage. During the season the plants were pruned by removing suckers close to the ground. The crop was inspected regularly (Appendix II). Insect-pests and diseases were controlled as necessary using standard chemicals.

3.5 Measurements

3.5.1 Compatibility of tomato scion x rootstock combinations

In order to determine the compatibility between the grafted cultivars, the survival rate of the grafted plants was measured. The surviving plants were assessed visually. Grafted plants that showed wilting after 7 days were classified as dead, hence not successful (compatible) scion x rootstock combinations. From each batch of 70 grafted plants the survival rate for each scion x rootstock combination was determined as follows:

$$S R (\%) = \frac{TNPF - NWS}{TNPF} \times 100$$

where:

SR = survival rate

TNPG = total number of plants grafted

NWS = number of wilted scions

3.5.2 Tomato growth parameters at the vegetative stage

In each plot (Cluster of 1 plant / plot x 8 pots), four plants were selected at random in order to measure several growth parameters (see below) starting at 21 days after transplanting to 90 days after transplanting. The four specific growth parameters that were measured were as follows:

- (i) plant height (PHT)
- (ii) stem diameter (SD)
- (iii) the number of branches (NB)
- (iv) the number of leaves per cluster (NLC).

The PHT was measured from the base of the stem to the apex of the main shoot. The SD was measured using veneer caliper. The NLC and the NB were counted manually three weeks after transplanting.

3.5.3 Tomato fruit quality and yield

Ripe tomato fruits were harvested twice per week sequentially over a period of eight weeks. For each plant, a sample of at least six randomly selected ripe fruits was used for determining fruit quality attributes which included:

- (i) total soluble solids (°Brix)
- (ii) titratable acidity (pH)
- (iii) fruit diameter (FD)
- (iv) fresh weight per plant (FW)
- (v) total fruit yield per plant (FY)

The fruit diameter was measured using veneer caliper. In order to determine the total soluble solids (Brix), juice of tomato fruits was extracted from the four fruits in a blender as described by Antoniali *et al.*, (2007). The homogenized sample was filtered through a filter into a beaker. The Brix value of the fruit juice was estimated using a refractometer (SMART-1, Model RFM-860) by placing a few drops of clear juice on the prism surface. The results were expressed as °Brix (%). The pH value of the juice was measured using a pH tester as described by Kitinija and Hussein (2005). In the method, the electrodes were immersed directly into the blended pulp and the results were read in 2-3 seconds.

3.6 Experimental design and statistical analysis

In the tunnel, each plot was constituted from a set of eight pots each containing one plant of a specific scion x rootstock combination (treatment). The experiment was laid out as a randomized complete block design (RCBD) replicated four times. All the quantitative data sets were subjected to the analysis of variance using the Genstat version 17.0 statistical software followed by mean separation using the Duncan multiple range test method.

4.0 CHAPTER FOUR: RESULTS

4.1 Grafting compatibility

The grafting of various tomato evaluation combination was successful averaging >90.0 % success rate per treatment (scion x rootstock combination). The highest success rate (97.0%) was observed in 'Rodade x Rodade' (R x R) and 'Money Maker x Money Maker' (MM x MM) (Fig 4.1). However after the seedlings were transferred to the tunnel, there was evidence of abnormal (delayed) compatibility between scion and rootstock (Fig 4.2). Nonetheless, these abnormalities did not result in mechanical weakening of the union at the adult plant stage. In addition, there were no plant suckers that developed since all the rootstocks were cut above the cotyledons.

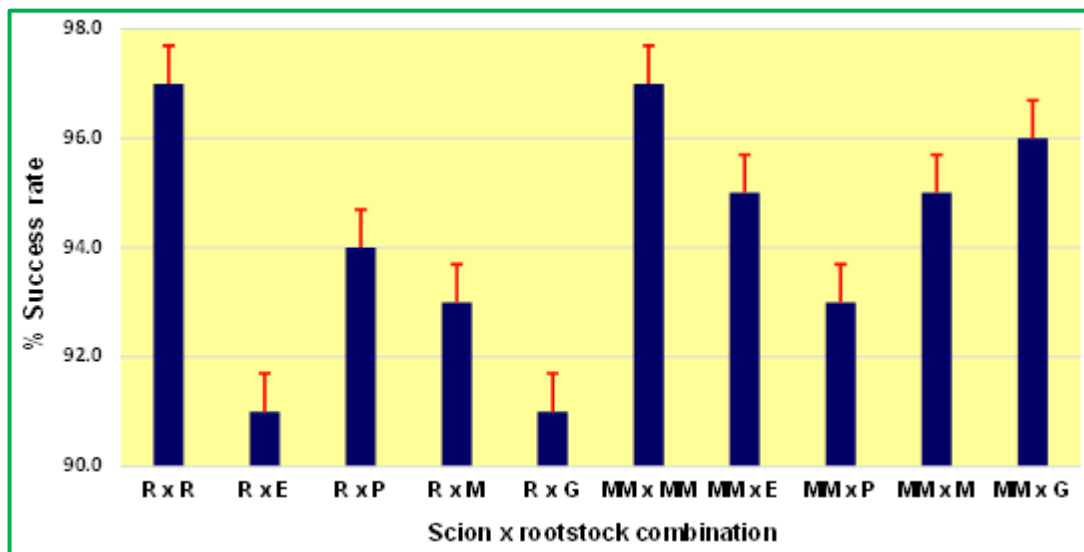


Fig. 4.1 The success rate of grafting various tomato scion x rootstock combinations.

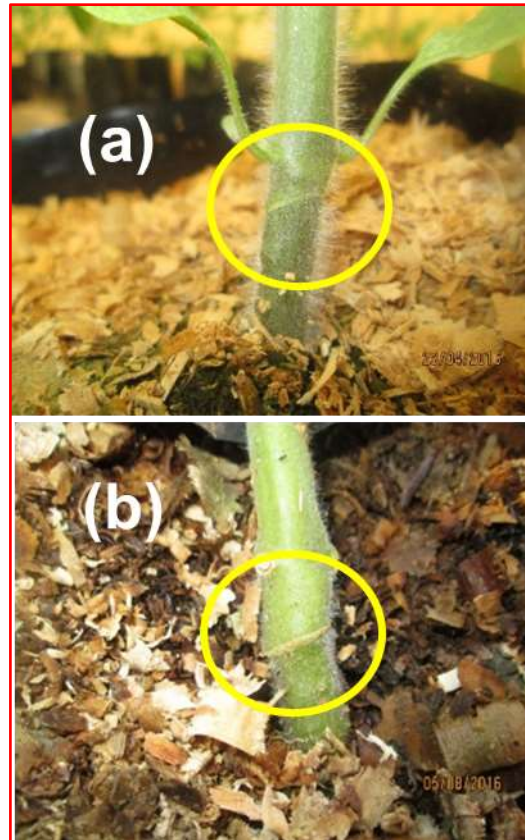


Fig. 4.2 The graft union in tomato indicating (a) normal compatibility between 'Rodade x Rodade' and (b) delayed (partial) compatibility between 'Rodade x Powapak'.

4.2 Impact of grafting on growth parameters

The analysis of variance (ANOVA) showed that there were highly significant ($P < 0.01$) differences among the scion x rootstock (SR) combinations for plant height (PHT) in the first season (Table 4.1). Similarly there were highly significant ($P < 0.01$) differences for all the four quantitative attributes including the time sampling (T) among the scion x rootstock combinations. In the second season, the stem diameter (SD) showed highly significant ($P < 0.01$) differences among the treatments. In addition, there were highly significant ($P < 0.01$) differences for T in all the four growth parameters namely PHT, SD, number of branches (NB) and the number of leaves per cluster (NLC) in the second season (Table 4.1). The treatment 'Rodade x Matterhorn' produced the tallest (49.06 cm) but 'Rodade x Powapak' produced the shortest (34.68 cm) plants (Table 4.2). Some individual treatments such as 'Rodade x Everest' showed some stunted plants which

possessed crinkled leaves (Fig 4.3) that were symptomatic of viral infection (Moriones and Navas-Castillo, 2000).

The average number of branches per plant (NB) and NLC in the trial was 10.43 and 61.12, respectively (Table 4.2). However, the SD and NB were similar among the treatments. The highest NLC (80.77) which was achieved by the control 'Moneymaker x Moneymaker' combination, was >30.0% higher than the trial average (61.12) and 28.0% more than the NLC that was attained by the 'Rodade x Rodade' combination (Table 4.2).

Table 4.1 Analysis of variance for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel at Thohoyandou (Limpopo Province).

Source	df	PHT (cm)	SD (mm)	NB	NLC
Season I					
Rep	3	556.81**	0.20	15.48**	2458.09
Scion-rootstock (SR)	9	227.77**	0.59	2.91	1845.08*
Rep x SR	27	129.12	0.61	1.08	656.15
Time of sampling (T)	2	18220.33**	280.75**	906.17**	66437.19**
SR x T	18	69.04	0.61	2.07	2412.73**
Season II					
Rep	3	1558.47**	2.72*	36.88**	1052.85**
Scion-rootstock (SR)	9	156.26	3.25**	16.52	251.31
Rep x SR	27	386.76**	1.79**	19.10**	493.19**
Time of sampling (T)	2	58832.67*	195.03**	1642.88**	34368.51**
SR x T	18	168.73	0.39	6.96	134.84

**; * = significant at the 1.0% and 5.0% probability levels, respectively. (PHT = plant height; SD = stem diameter; NB = number of branches per plant; NLC = number of leaves per cluster).

Season I = 2015

Season II = 2016

Table 4.2 Means for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel during 2016 at Thohoyandou (Limpopo Province).

Scion x rootstock combination (Code)	PHT (cm)	SD (mm)	NB	NLC
Rodade x Matterhorn (5)	49.063 a	6.72 a	10.71 a	75.25 ab
Money Maker x Money Maker (6)	43.6 ab	6.55 a	11.17 a	80.77 a
Rodade x Rodade (4)	42.6 ab	6.71 a	10.65 a	62.79 abcd
Money Maker x Golf (10)	42 ab	6.66 a	10.94 a	68.52 abcd
Money Maker x Money Maker (8)	39.75 ab	6.47 a	10.27 a	70.19 abc
Rodade x Matterhorn (3)	38.52 b	6.25 a	9.67 a	48.02 de
Rodade x Everest (1)	37.69 b	6.44 a	10.04 a	42.44 e
Money Maker x Golf (9)	36.77 b	6.5 a	9.92 a	54.08 bcde
Money Maker x Powapak (7)	35.69 b	6.34 a	10.94 a	55.71 bcde
Rodade x Powapak (2)	34.69 b	5.97 a	10.19 a	53.45 cde
Grand total	40.03	6.45	10.43	61.12
C.V. (%)	22.81	11.72	13.37	49.07
R ² (%)	89.97	94.55	94.34	80.15

Means followed by different letters are significantly different at the 5.0% probability level. (PHT = plant height; SD = stem diameter; NB = number of branches per plant; NLC = number of leaves per cluster).



Fig. 4.3 Individual plants were stunted and possessed crinkled leaves suggesting viral infection in some of the tomato scion x rootstock combinations that were evaluated in a tunnel at Thohoyandou (Limpopo Province).

In the second season, the height of the plants was similar among the treatments but the plants were generally taller than in the first season (Fig 4.4) with some plants reaching the roof of the tunnel (Fig. 4.5). The ANOVA for growth parameters for the combined two seasons indicated highly significant ($P < 0.01$) differences due to season in all the four growth parameters (Table 4.3). In addition, there was a highly significant ($P < 0.01$) interaction between the season x scion – rootstock combination. Similarly, highly significant ($P < 0.01$) main effects of time of sampling as well as season x time of sampling interaction were observed (Table 4.3). The season x scion – rootstock x time of sampling interaction was highly significant ($P < 0.1$) only for NLC (Table 4.3).

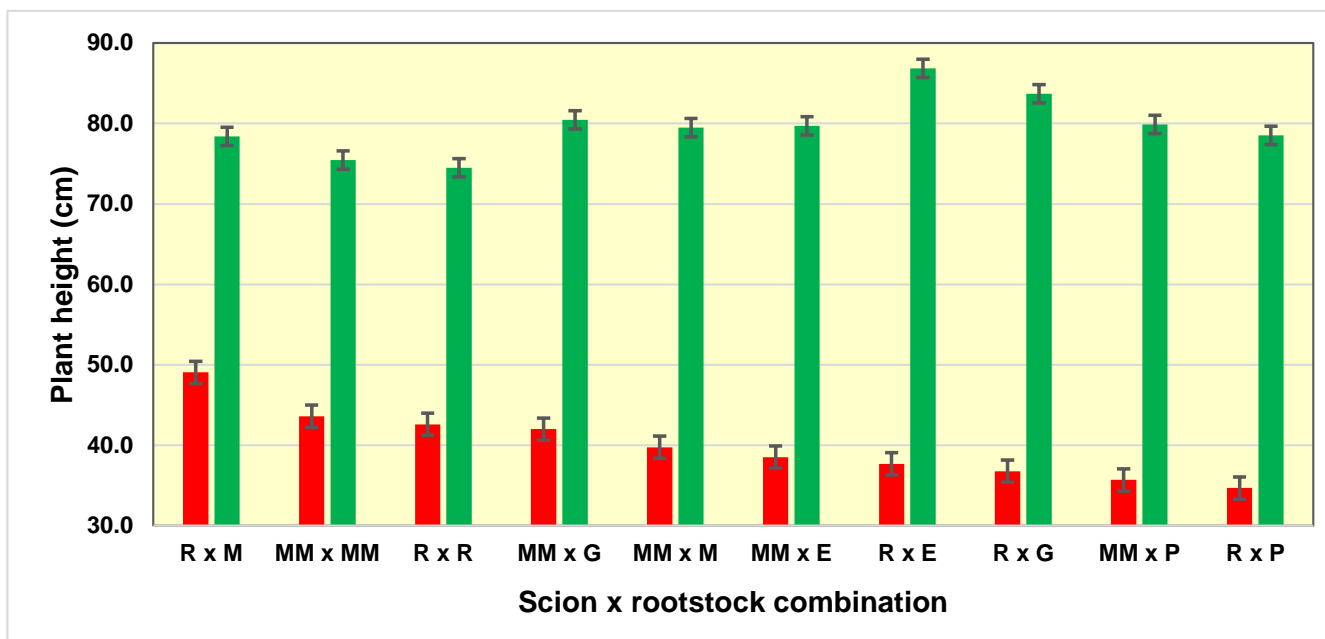


Fig. 4.4 Seasonal variation in plant height among tomato scion x rootstock combinations that were evaluated in a tunnel during 2016 (red bars) and 2017 (green bars) at Thohoyandou (Limpopo Province). (R x M = Rodade x Matterhorn; MM x MM = Money Maker x Money Maker; R x R = Rodade x Rodade; MM x G = Money Maker x Golf; MM x M = Money Maker x Matterhorn; MM x E = Money Maker x Everest; R x E = Rodade x Everest; R x G = Rodade x Golf; MM x P = Money Maker x Powapak; R x P = Rodade x Powapak).



Fig. 4.5 Some of the tomato scion x rootstock combinations were markedly tall, reaching the roof of the tunnel at Thohoyandou (Limpopo Province).

Table 4.3 Combined analysis of variance for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel for two seasons at Thohoyandou (Limpopo Province).

Source of variance	df	PHT (cm)	SD (mm)	NB	NLC
Season (Sn)	1	94449.11 **	1071.79**	3228.35**	22074.07**
Rep	6	1057.64 **	1.46	26.18**	1755.47**
Scion-rootstock (SR)	9	107.58	2.93**	9.16	582.36
Sn x SR	54	276.33 *	0.89	10.27	1514.02**
Rep x SR(Sn)	2	257.94 **	1.20**	10.09**	574.68
Time (T)	2	70451.91**	471.11**	2494.67**	93071.46**
Sn x T	2	6601.09 **	4.67**	54.39**	7734.24**
SR x T	18	104.71	0.35	4.57	963.92*
Sn x SR x T	18	133.05	0.64	4.44	1583.66**

**; * = significant at the 1.0% and 5.0% probability levels, respectively. (PHT = plant height; SD = stem diameter; NB = number of branches per plant; NLC = number of leaves per cluster).

In terms of the number of leaves per cluster, a considerable difference due to season was observed in both 'Money Maker x Everest' and 'Rodade x Everest' (Fig 4.6). In contrast, the combination 'Rodade x Moneymaker' as well as 'Moneymaker x Moneymaker' showed no significant seasonal fluctuation for the number of leaves that were developed per cluster. For the scions that were grafted on 'Rodade', the highest (32.4%) positive change (or increment) in the NLC relative to the control ('Rodade x Rodade') occurred in 'Rodade x Everest' (Fig. 4.7). On average, the NB showed a generally depressed but positive change relative to the control while PHT showed a moderate positive change. Similarly, for the scions that utilized 'Moneymaker' as the rootstock, the highest (40.54%) positive change in the NLC relative to the control ('Moneymaker x Moneymaker') was achieved by 'Moneymaker x Everest' (Fig. 4.8). Over the two seasons, the average PHT and NB were 59.87 cm and 14.1 cm respectively (Table 4.4).

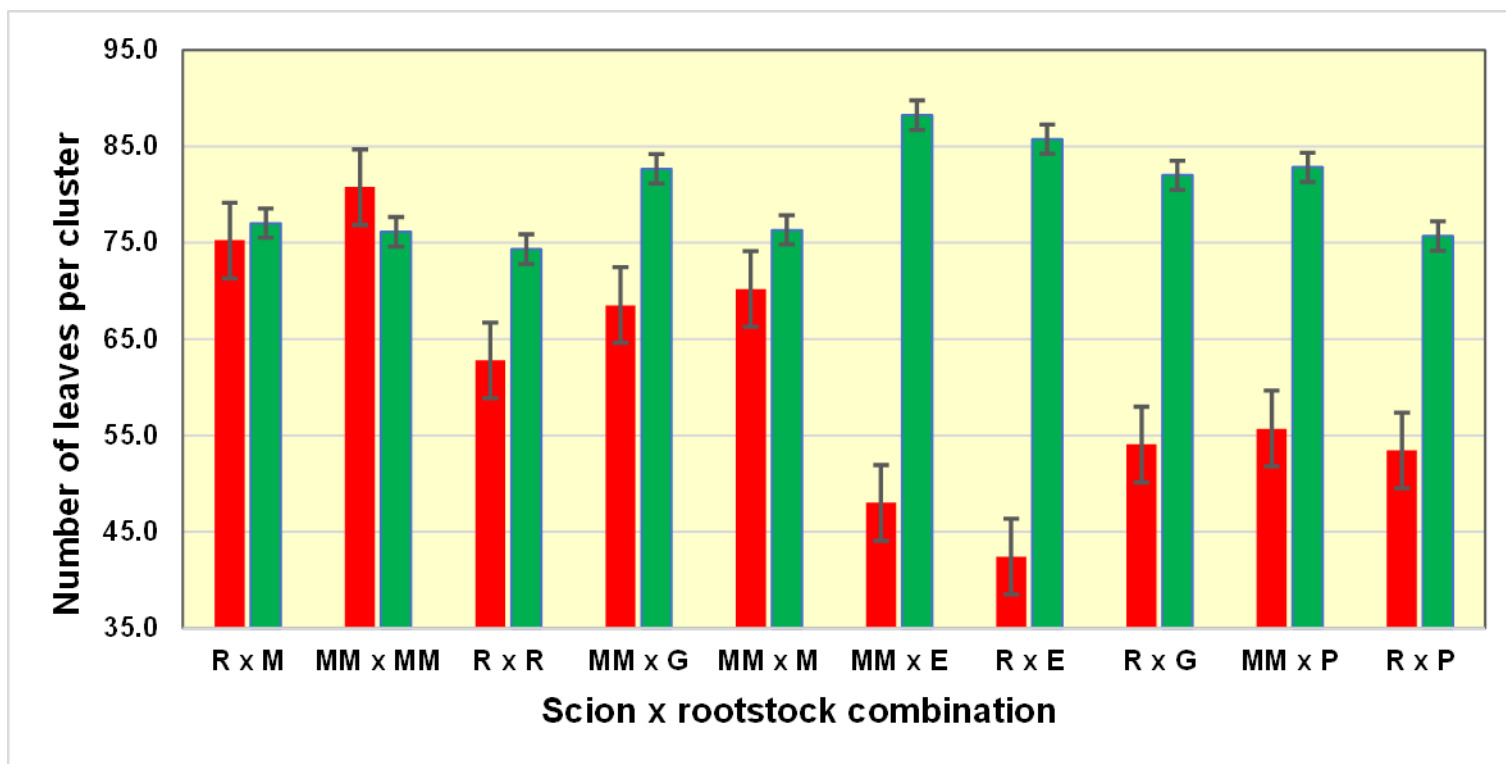


Fig. 4.6 Seasonal variation in the number of leaves per cluster among tomato scion x rootstock combinations that were evaluated in a tunnel during 2016 (red bars) and 2017 (green bars) at Thohoyandou (Limpopo Province). (R x M = Rodade x Matterhorn; MM x MM = Money Maker x Money Maker; R x R = Rodade x Rodade; MM x G = Money Maker x Golf; MM x M = Money Maker x Matterhorn; MM x E = Money Maker x Everest; R x E = Rodade x Everest; R x G = Rodade x Golf; MM x P = Money Maker x Powapak; R x P = Rodade x Powapak).

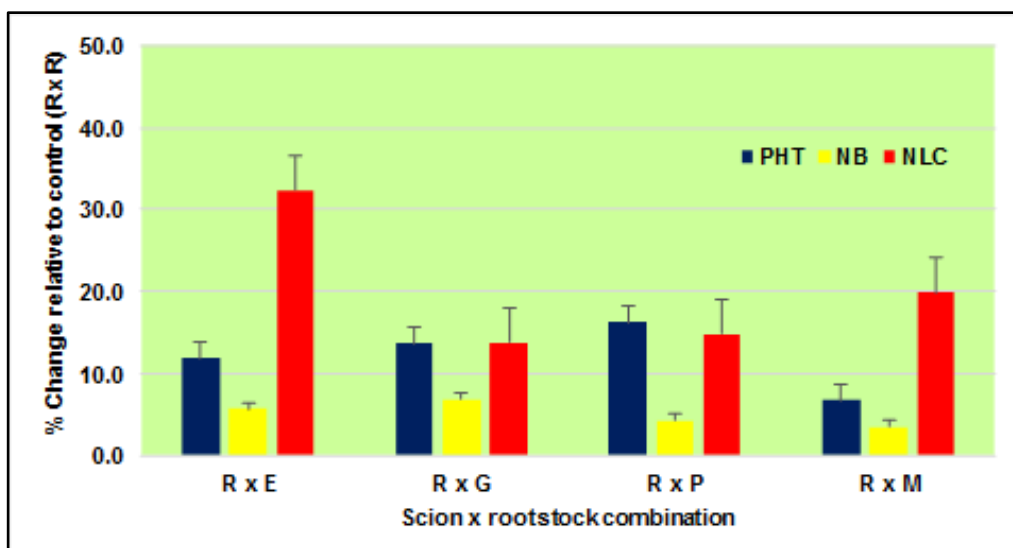


Fig. 4.7 The magnitude of increment in three growth parameters among tomato cultivars that were grafted on a 'Rodade' rootstock and subsequently evaluated in the tunnel at Thohoyandou (Limpopo Province). (R x E = Rodade x Everest; R x G = Rodade x Golf; R x P = Rodade x Powapak; R x M = Rodade x Matterhorn).

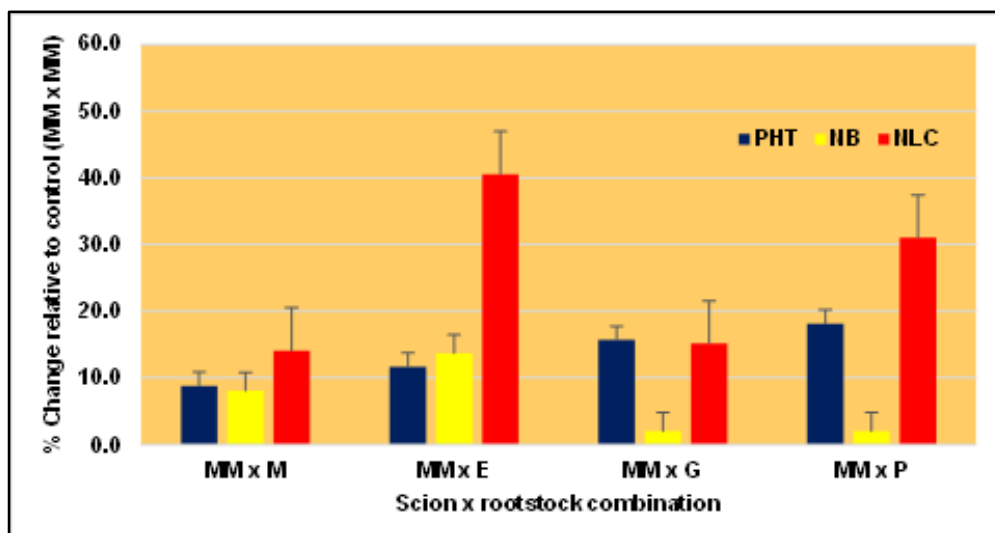


Fig. 4.8 The magnitude of increment in three growth parameters among tomato cultivars that were grafted on a 'Money Maker' rootstock and subsequently evaluated in the tunnel at Thohoyandou (Limpopo Province). (MM x M = Money Maker x Matterhorn; MM x E = Money Maker x Everest; MM x G = Money Maker x Golf; MM x P = Money Maker x Powapak).

Table 4.4 Means from the combined analysis of variance for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel for two seasons at Thohoyandou (Limpopo Province).

Scion x rootstock combination (Code)	PHT (cm)	SD (mm)	NB	NLC
Rodade x Matterhorn (5)	63.738 a	8.67 b	14.49 a	76.14 a
Money Maker x Money Maker (6)	59.53 a	8.4 bc	14.34 a	78.45 a
Rodade x Rodade (4)	58.56 a	9.32 a	13.87 a	69.56 a
Money Maker x Golf (10)	61.22 a	8.75 ab	14.01 a	75.59 a
Money Maker x Money Maker (8)	59.62 a	8.62 b	13.87 a	73.26 a
Rodade x Matterhorn (3)	59.11 a	8.34 bc	13.87 a	68.13 a
Rodade x Everest (1)	62.32 a	8.51 bc	14.14 a	64.09 a
Money Maker x Golf (9)	60.23 a	8.59 b	14.65 a	68.04 a
Money Maker x Powapak (7)	57.79 a	8.5 bc	15.16 a	69.27 a
Rodade x Powapak (2)	56.61 a	7.92 c	12.92 a	64.57 a
Grand mean	59.87	8.56	14.1	70.71
C.V. (%)	18.75	9.59	16.69	32.85
R ² (%)	94.81	96.36	93.36	83.58

Means followed by different letters are significantly different at the 5.0% probability level. (PHT = plant height; SD = stem diameter; NB = number of branches per plant; NLC = number of leaves per cluster).

4.3 Impact of grafting on fruit productivity and quality parameters

The fruit productivity and time to ripening varied within individual plants among the treatments (Fig. 4.9). The highest fruit yield per plant (4.0 kg) was observed for 'Money Maker x Everest'. The scion x rootstock combination between 'Rodade x Rodade' attained the lowest (1.3 kg) fruit yield. However, the observed differences in the means of all the productivity and quality parameters were not significant ($P < 0.05$) (Appendix III). Nonetheless, each genotype produced approximately five tomato fruits across the seasons. The highest fruit fresh weight was obtained by the 'Money Maker x Golf' combination (Fig. 4.9). The means of Brix and pH values among the genotype were 4.64 and 4.14 respectively while 'Moneymaker x Everest' produced the highest fruit number (FN) per plant (7.47) (Appendix III). A narrow pH range (4.11-4.17) was observed among the treatments. The grafting combination between 'Money Maker x Golf' produced the highest (136.0g) fruit fresh weight (Fig 4.10) as well as the highest (5.01%) Brix value (Appendix III).

4.4 Correlation between growth production and quality traits of grafted tomatoes

A highly significant ($P < 0.01$) positive correlation between fruit number and plant height as well as the number of leaves per cluster (Table 4.5). At least 60.0% of the variation in fruit load per plant was explained by the change in the number of leaves per cluster (Fig. 4.11).



Fig. 4.9 Variation in fruit load, fruit size, time to ripening within individual plants among grafted tomato plants that were evaluated in a tunnel at Thohoyandou (Limpopo Province).

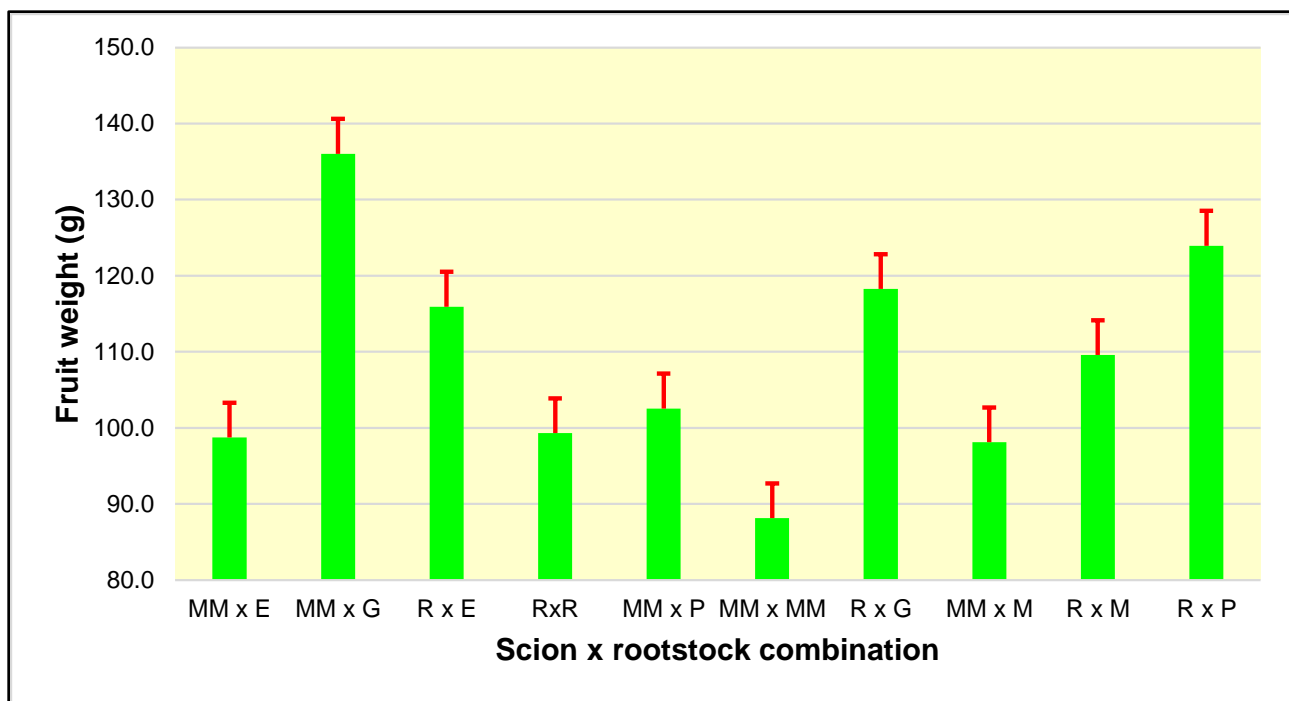


Fig. 4.9 Variation in total fruit fresh weight among grafted tomato plants that were evaluated in a tunnel at Thohoyandou (Limpopo Province). (MM x E = Money Maker x Everest; MM x G = Money Maker x Golf; R x E = Rodade x Everest; R x R = Rodade x Rodade; MM x P = Money Maker x Powapak; MM x MM = Money Maker x Money Maker; R x G = Rodade x Golf; MM x M = Money Maker x Matterhorn; R x M = Rodade x Matterhorn; R x P = Rodade x Powapak).

Table 4.5 Pearson correlation coefficients among quantitative traits of grafted tomato plants that were evaluated in a tunnel at Thohoyandou (Limpopo Province).

	PHT	SD	NB	NLC	FN	FW	FY	FD	Brix	pH
PHT	1.0000									
SD	0.2965	1.0000								
NB	0.333	0.2938	1.0000							
NLC	0.3582	0.2812	0.2955	1.0000						
FN	0.7020**	0.4474	0.3137	0.7751**	1.0000					
FW	-0.3077	-0.1792	0.3121	0.0173	-0.149	1.0000				
FY	0.2037	0.1102	0.4881	0.4333	0.6449	0.4467	1.0000			
FD	-0.3647	0.593	-0.2773	-0.0964	0.0100	0.2421	0.3122	1.0000		
Brix	-0.1364	-0.3165	-0.2408	0.5785	0.2069	0.3533	0.0411	-0.1578	1.0000	
pH	0.5917	-0.1446	0.3137	0.2061	0.0332	0.0332	0.0113	-0.4874	0.2657	1.0000

** = significant at the 1.0% probability level. (PHT = plant height; SD = stem diameter; NB = number of branches per plant; NLC = number of leaves per cluster; FN = fruit number per plant; FW = fresh fruit weight per plant; FY = total fruit yield per plant; FD = fruit diameter; FYP = total fruit yield per plant; pH = titratable acidity; Brix = titratable soluble solids).

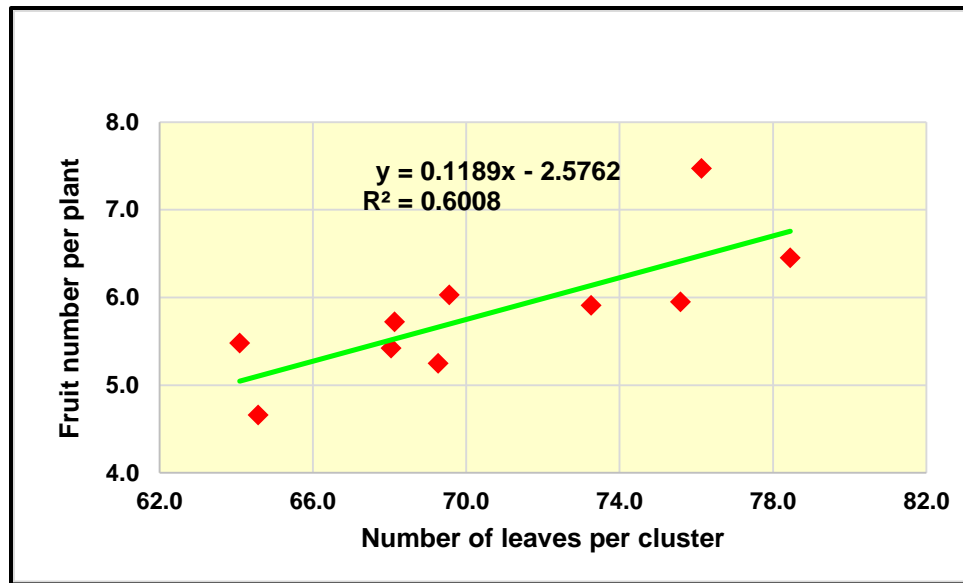


Fig. 4.11 The relationship between fruit number per plant and the number of leaves per cluster among various scion x rootstock combinations of tomato cultivars that were evaluated under tunnel conditions at Thohoyandou.

5.0 CHAPTER FIVE: DISCUSSION

The results showed that the selected scion x rootstock combinations were compatible for grafting although there were a few cases of delayed compatibility. These findings were in agreement with several similar studies that used distinct tomato cultivar combinations in grafting (Di Gioia *et al.*, 2015; Khah *et al.*, 2006; Andrew and Marquez, 1993). Incompatibility was previously attributed to poor vascular connection between rootstock and scion which impedes water uptake and salt translocation (Santa-Cruz *et al.*, 2002; He *et al.*, 2009; Flores *et al.*, 2010) as well as nutrient uptake (Torii *et al.*, 1992), thus interfering with several physiological characteristics (Rodrigues *et al.*, 2001; Martínez-Ballesta *et al.*, 2010). In some perennial species, incompatibility was attributed to a reduction in auxin and lignification which was associated with high phenols (Pina *et al.*, 2012).

The high success rate of the grafted tomato plants reported in this study confirmed that the method can be used successfully and routinely in tomato production systems. It also suggested that there was no viral infection in the grafted plant material since this was also cited as one of the potential causes of incompatibility (Rowhani *et al.*, 2016; Golino, 1993). However, this can be solved by using virus free plant material and maintaining a high level of propagation skill. In addition, the high tomato plant survival rate (100.0%) was also reported recently in a study that used the cleft graft method (Zeist *et al.*, 2017; Msogoya and Mamiro, 2016). In particular, the grafting method is useful where resistance to soil-borne diseases is required in susceptible genetic backgrounds that are otherwise preferred by end-users. For instance, tomato cultivars were successfully grafted on eggplant rootstocks that are resistant to soil borne diseases (Msogoya and Mamiro, 2016).

The results of this study also indicated that some of the growth parameters at the vegetative growth stage essentially remained intact (or unchanged) which is desirable particularly if the grafting is intended only for combating diseases without altering the characteristics of the scion genotype. However, in the case of the number of leaves per cluster, the results showed that the selection of the parental combination was important since there was evidence for improvement in this attribute particularly in 'Rodade x Everest' and 'Money Maker x Everest' grafting combinations. Therefore, where an improvement in the number of clusters per plant is required, the selection of specific parental combinations could be useful. The number of leaves per cluster

in this study was also significantly correlated to the fruit number, hence fruit yield. According to Papadopoulos and Pararajasingham (1997), the balance between the vegetative and reproductive attributes is important because photosynthesis promotes fruit set. Probably in future, there will be merit in determining the leaf angle also since it influences the plant canopy and hence light absorption efficiency (Falster and Westoby, 2003).

The observed few individual stunted plants were likely due to viral infection since the leaf symptoms in particular resembled those of viral attack in tomato plants (Moriones and Nava-Castillo, 2000). Under tunnel or controlled conditions, viral attacks (transmitted by aphids or whiteflies) on tomato have been reported previously. One of the solutions to minimize viral diseases in such closed environments requires the use of UV-absorbing polyethylene films or with UV-absorbing nets (Antignus *et al.*, 2001; Antignus, 1999; Antignus *et al.*, 1996a) which can filter out the light spectrum between 280–380 nm, thus significantly reducing infestation of crops by whiteflies, aphids and thrips (Antignus *et al.*, 1996b, 1998). In a study that raised tomatoes in a UV-absorbing greenhouse, the crop was protected against whitefly-borne viruses (Monci *et al.*, 2002). At least the technology may be worthwhile employing in commercial tomato production situations in Limpopo Province.

The significant seasonal effect that was observed on some of the growth and productivity attributes indicated the importance of taking into consideration environmental factors in tomato production. While for instance the growth medium and moisture were controlled effectively under tunnel conditions, the temperature effects were not controlled adequately. The impact of temperature on the growth of tomato was reported widely in previous studies involving tomato plants (Dinar and Rudich, 1985). Nonetheless, in this study, there were no extreme temperatures at flowering for instance that could lead to flower abortion (Abdalla *et al.*, 1968) hence reduce fruit yield. The fruit yield as indicated by the fruit number per plant and total fruit yield, was within the expected range which validated the viability of grafted tomatoes under tunnel conditions at the location.

The results also showed that there were no significant differences between the control and the treatment for both productivity indicators and fruit quality attributes in tomato. A negative influence

of grafting on these parameters would otherwise render the method uneconomical, hence not preferred widely by researchers. Equally important, was that the results suggested that the time of production in a given season is important for optimizing productivity. The significant interaction between sampling time and genotype suggested that tomato producers need to take into consideration both the time of cultivation and sampling (or harvesting) the fruit.

The results also indicated clearly that both the Brix and pH of tomato were not influenced by grafting. The pH showed a narrow range of values suggesting that this quality trait does not fluctuate widely. Therefore, some fruit quality attributes are unlikely to be affected by the grafting technique. In a similar study, total soluble solids were not significantly affected by grafting (Gioia *et al.*, 2015; Khah *et al.*, 2006). In summary, the grafting method in tomato influenced only a few growth parameters but did not influence the fruit chemical quality.

6.0 CHAPTER SIX: CONCLUSIONS AND RECOMMENDATIONS

The results of this study showed a high plant survival rate of grafted tomato plants. This was in agreement with several other previous studies involving tomato grafting. The high success rate partly suggested that there was no viral infection in the grafted plant material. Among the vegetative growth parameters, the number of clusters per plant showed marked improvement in some grafting combinations particularly in 'Rodade x Everest' and 'Money Maker x Everest' suggesting that the selection of specific parental combinations could be useful in tomato grafting work. There were some few stunted plants among the grafted plants possibly due to viral infection in the tunnel. Therefore, it is recommended that the structure of tunnels especially under large-scale tomato production in the area, utilize UV-absorbing polyethylene films or with UV-absorbing nets which can filter out the UV light thus reducing infestation of crops viral vectors such as whiteflies, aphids and thrips.

A significant seasonal effect was observed on some of the growth and productivity attributes indicating that environmental factors need to be taken into consideration in tomato production. The fruit yield was within the expected range which validated the viability of producing grafted tomatoes under tunnel conditions at the location. However, grafting did not influence significantly chemical fruit quality attributes in tomato. The pH showed a narrow range of values. A significant interaction between sampling time and genotype suggested that tomato producers need to take into consideration both the time of cultivation and harvesting the fruit.

In future, there will be merit in including a wider range of tomato germplasm in order to validate the observations under the agro-ecological conditions that are represented by the study location. The range of vegetative growth parameters, yield attributes and fruit quality attributes could also be increased to include among others, leaf angle, leaf morphology, leaf area, sensory properties, vitamin content, carotene and lycopene content, mineral profiles as well as the shelf life of the fruits. In addition, it will be useful to identify the tomato cultivars that are susceptible to soil-borne diseases prevalent in the area in order to deliberately use the grafting method to circumvent the disease. It will also be useful to evaluate the grafted plants under field conditions since some of the end-users, particularly smallholder farmers are constrained by the lack of infrastructures such as the tunnels. The generality of farmers tend to produce tomato under rain-fed conditions.

Therefore, the response of these grafted tomatoes to moisture stress could be useful in order to identify elite scion-rootstock combinations.

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APPENDICES

Appendix I

Composition of a 25.0 kg bag of fertilizer that was utilized for raising grafted tomato plant in the tunnel.

	<u>Element</u>	<u>Quantity</u>
<i>Granule Haifa Cal fertilizer</i>		
(i)	Nitrogen (N)	15.5 %
(ii)	Nitrate-nitrogen (N-NO ₃)	14.4 %
(iii)	Ammonium-nitrate (N-NH ₄)	1.1 %
(iv)	Calcium-oxide (CaO)	26.5 %
(v)	Calcium (Ca)	19.0 %
<i>Haifa Solution Hydroponic Mix</i>		
(i)	Nitrogen (N)	64.0 g/kg
(ii)	Phosphorus (P)	43.0 g/kg
(iii)	Potassium (K)	238.0 g/kg
(iv)	Magnesium (Mg)	26.0 g/kg
(v)	Sulphur (S)	30.0 g/kg

Appendix II

Crop inspection in the tunnel was conducted regularly.



Appendix III

Means for growth parameters of tomato scion x rootstock combinations that were evaluated in a tunnel during 2017 at Thohoyandou (Limpopo Province).

Scion x rootstock combination (Code)	PHT (cm)	SD (mm)	NB	NLC
Rodade x Everest (1)	86.87 a	10.58 a	18.25 a	85.75 a
Rodade x Golf (9)	83.69 a	10.69 a	19.47 a	82.00 a
Money Maker x Golf(10)	80.44 a	10.84 a	19.38 a	82.67 a
Money Maker x Powapak (7)	79.89 a	10.67 a	19.47 a	82.83 a
Money Maker x Everest (3)	79.70 a	10.43 a	18.08 a	88.24 a
Money Maker x Matterhorn (8)	79.49 a	10.77 a	17.05 a	76.33 a
Rodade x Powapak (2)	78.54 a	9.88 a	15.67 a	75.69 a
Rodade x Matterhorn (5)	78.41 a	10.71 a	18.29 a	77.02 a
Money Maker x Money Maker (6)	75.46 a	10.26 a	17.53 a	76.14 a
Rodade x Rodade (4)	74.51 a	11.91 a	14.75 a	74.33 a
Grand mean	79.71	10.68	17.77	80.3
C.V. (%)	16.29	8.26	17	16.71
R ² (%)	93.13	91.18	88.42	89.26

Means followed by the same letter in the same column are not significantly different at the 5.0% probability level.