ROOTSTOCK-SCION GENOTYPE AND ENVIRONMENT INTERACTION IN A SOUTH AFRICAN CITRUS BREEDING PROGRAMME

by

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Author's declaration

I hereby declare that I am the sole author of this thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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Zelda Bijzet

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Dedication

I dedicate this thesis to my deceased parents Wouter en Tossie Marais who could not witness this achievement. They are greatly acknowledged for teaching me to believe in God, in myself, and in my dreams.

The formulation of a problem is often more essential than its solution, which may be merely a matter of mathematical or experimental skill.

Einstein, Albert

"An approximate answer to the right problem is worth a good deal more than an exact answer to an approximate problem."

John Tukey

TABLE OF CONTENTS

List of figures

List of tables

Chapter 1

GENERAL INTRODUCTION AND PROBLEM STATEMENT

Common knowledge regarding man's earliest relationship with fruit trees is attained from Biblical references. However, some believe that man's relationship with fruiting plants began long before the origin of agriculture in 8000-10 000 BC, when all human beings were either hunters or gatherers. Fruits gathered from the wild probably formed the core of the human diet, being excellent sources of fibre, vitamins, and other nutritious compounds. Domestication of wild fruiting plants probably originated from seeds dumped at the edge of villages. As our early ancestors evolved from the process of nomadic food gathering to developing permanent food sources, crop breeding became an established practice (Khan and Kender, 2007).

Today, fruit crops are important agricultural commodities, contributing to the global economy as well as being a major source of income for developing countries. Worldwide, millions of hectares of land have been devoted to its' production, and the livelihood of literally millions of farming families depends on continued global trade. Due to well-established world trade networks, as well as sophisticated cultural and postharvest technologies, fruits can be enjoyed worldwide throughout much of the year, instead of mere weeks per year like our ancestors experienced. However, the pressure associated with global trade as well as the competitive international market, the demand for high quality fruit by consumers, the strong pressure to reduce chemical use, and a need to enhance the economic efficiency of production, compel tree-fruit growers to find alternative, economically and environmentally sustainable production practices.

Consumer demand, especially for fresh food products, has increased dramatically in recent years driven by growing average incomes globally as well as by a more informed society (Mashinini, 2006; Von Braun, 2007). A more informed society is aware of the health benefits of fresh fruits and vegetables with regard to the incidence of, amongst others, cancer, cardiovascular diseases and neurological degeneration. According to Mashinini (2006) food retailers are making considerable investments to meet consumers' demands by stocking more healthy, nutritious and convenient products that suit today's consumer lifestyle. The role that research and development units should play with the changing society cannot be overemphasized because some of these recent changes need scientific research support. The hundreds of fruits, vegetables, and grains that are now found on supermarket shelves are the results of plant breeders (Khan and Kender, 2007).

Following apple and banana, citrus is the third most important fruit crop in the world and accounts for a production of about 115 million tons with an area of cultivation spread over 8.6 million hectares. Although South Africa ranks 18th on production and number of hectares planted, South Africa is one of the top three exporting countries of citrus in the world.

Commercial citrus trees are two different but compatible genotypes that are combined through budding to form a compound genetic unit (Koepke and Dhingra, 2013). This composite genotype is formed through budding a single bud (refer to as a scion) onto a rootstock to form a commercial important compound genetic unit with a significant scientific interest (Rogers and Beakbane, 1957). For the purpose of this thesis, this compound genetic unit or two-part tree will be referred to as a "stion," which derives from stock + scion (Hume, 1957).

The importance of a citrus rootstock rests on the subtle distinction between general reasons why rootstocks are used and individual rootstock characteristics. In citrus, rootstocks are used for true-to-type propagation of mono-embryonic scion cultivars. The citrus types that can be produced as true-to-type nucellar seedling trees are prone to extensive juvenile (time to bearing) periods as well as excessive tree vigour and are therefore rather grafted onto rootstocks to control/manage the juvenile period and tree size. The degree of nucellar embryony within rootstock cultivars that is related to ease, expense, and consistency of propagation are also important rootstock nursery traits. It is thus not surprising that propagation of citrus trees with rootstocks has long been preferred over the use of scion cuttings taken from mature trees.

Specific traits or individual characteristics of rootstocks, which contribute in positive ways to the performance of a citrus tree, include those that influence various horticultural traits. It can provide tolerance to pests and diseases and certain soil and site conditions that contribute significantly to orchard profitability. It is thus evident from the above that the evaluation of new scion cultivars cannot be done without grafting it onto a rootstock and more importantly, the performance of a rootstock cultivar can only be derived by measuring the attributes of the scion such as yield and quality of the fruits produced, thus making rootstock-scion interactions of critical importance. Hume (1957) stated that: ‘‘*No problem in citrus culture is worthier of painstaking research than the one having to do with rootstocks. The whole gamut of citrus fruit production is affected by the relation of rootstock to scion and the adaptability of different combinations to the environment. Something is known but much remains to be found out*.’’ Physiological reactions and disease reactions are relatively specific in relation to stionic combinations used, and new hybrids must be evaluated for these reactions (Hume, 1957).

A grafted or budded plant can produce growth patterns and reactions which may be different from what would have transpired if each part of the stion were grown separately or when the scion was grafted or budded onto different rootstocks. Some of these reactions can have major horticultural value. This varying influence of a rootstock on the performance of a scion cultivar or vice versa is known as "stock scion relationship" (Rogers and Beakbane, 1957).

In farming with a fruit tree, the deployment of a new scion and especially a new rootstock is a long-term commitment associated with huge financial inputs. The value and impact of the new genotype (scion or rootstock) is only evident once in bearing which is a minimum of two years after planting. Break-even is usually only after eight years of production. It is thus paramount that a well-informed choice of stion based on scientific values is made. For instance, citrus industries globally will benefit from genetic improvements leading to the release of superior rootstock and scion cultivars (Gmitter *et al*. 2007).

For this purpose, rootstock-scion trials have been part-and-parcel of breeding programmes worldwide. According to Gmitter *et al*. (2007) many breeding programmes have been inefficient due to a lack of genetic knowledge of important traits, incomplete understanding of the consequence of taxonomic differences and relationships as well as various breeding constraints such as poly-embryony and juvenility. In this regard, the ARC-ITSC's citrus breeding programme was no exception, therefore a more structured, and targeted breeding programme has been advocated by Breedt *et al*. (1996). Figure 1.1 illustrates the breeding process that has been applied for citrus at the ARC-ITSC.

It can be seen from Figure 1.1 that even breeding populations are not evaluated on their own roots. The reason for this is two-fold, the first being susceptibility of the scion to soil borne diseases and secondly due to growth patterns and reactions due to rootstock-scion relationships and which will become important during vegetative propagation of a new beneficial genotype.

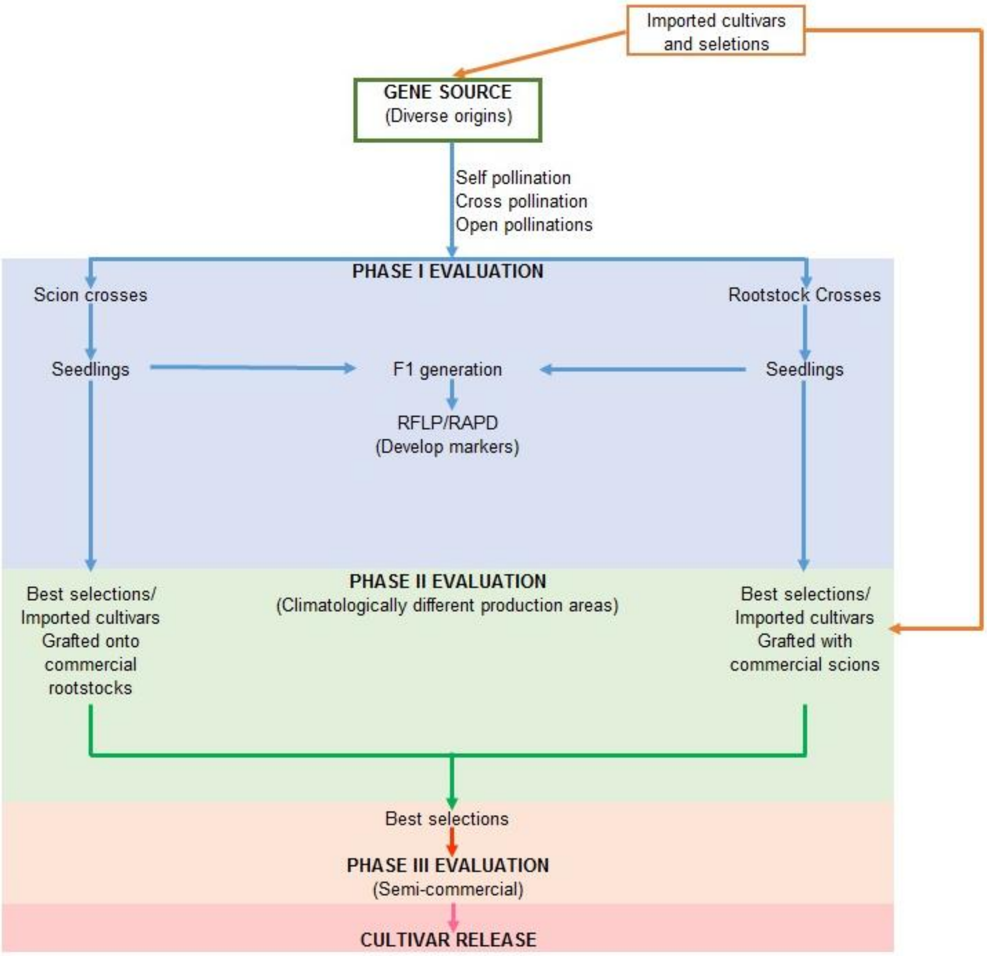

Figure 1.1 The scion breeding process implemented by the ARC-ITSC since 1992 (Breedt *et al*., 1996)

Layout of fruit evaluation trials differ from breeding programme to breeding programme world-wide with no specific model available. However, replicated field trials for horticultural evaluation is an integral part of all breeding programmes (Castle, 1995; Breedt *et al*., 1996; Gmitter *et al*., 2007, Kahn *et al.*, 2007). Variation in trial layouts renders the data and recommendations are applicable only to specific trials. According to Shaner *et al.* (1982) and Hildebrand and Poey (1985) regional yield trials are networks of experiments by which a set of cultivars is usually assessed to make genotype recommendations. In this context, trials typically: are research-managed; comprise out of six to 15 genotypes; are conducted in five to 10 localities; and are laid out in a randomized complete block design with two to four replicates, with more complex designs sometimes adopted.

However, in tree-crops, where a stion is involved, the interaction of rootstock and scion genotypes with each other as well as each individually with the environment should be taken into account. Due to rootstock scion interaction, it is advisable to use a range of rootstocks to ensure the best combination. With regard to rootstock selections with, for instance improved dwarfing qualities and diseases resistance, producers would want to know the relevance of rootstock selections to their environment, budded or grafted with their particular scion cultivar. It should also be mentioned that fruit yield and some other quality traits in woody plants are metric traits whose phenotypic expression in individual trees may vary between seasons as a response to changes in the environment or due to measurement of error variance. However, these traits are evaluated more than one time in the same individual over several seasons, such as fruit production and it is possible to estimate a repeatability coefficient.

The broad goal of this study was to successfully separate the genotype (G) and genotype by environment interaction (GEI) of the stion in a scion and a rootstock G and GEI. The envisaged application of the study is to provide a statistical method for time and cost effective evaluation of promising selections in a South African citrus breeding programme, taking into account the interaction of scion genotypes and the environment (localities/years), rootstock genotypes and the environment and the interaction between scion and rootstock genotypes.

The primary objectives of this study were:

1) to use data from previous Phase II citrus trials at the ARC-ITSC to study cultivar evaluation based on genotype main effect, analysed by univariate statistical analysis methods
2) to quantify GEI for yield and quality with regard to rootstocks grafted to very different citrus scion types
3) to separate stion GEI into a scion GEI and a rootstock GEI for yield and quality per citrus scion type
4) to differentiate scion G and GEI as well as rootstock G and GEI from that of the stion

5) to explore the relevance of additive main effects and multiplicative interaction (AMMI) and genotype plus genotype-by-environment interaction (GGE) biplots to investigate G, E and GEI amongst citrus scions, rootstocks and environments in the traditional citrus scion Phase 2 trial layout
6) to integrate the knowledge into an economic and time efficient evaluation protocol for citrus scions and rootstocks to be implemented in the current breeding programmes at the ARC-ITSC.

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Chapter 2

GENOTYPE AND ENVIRONMENT INTERACTION IN FRUIT TREES RELATING TO SCION AND ROOTSTOCK WITH CITRUS SPP AS REFERENCE: A LITERATURE REVIEW

2.1 INTRODUCTION

According to Mudge *et al*. (2009) nomadic peoples in the Fertile Crescent, 10 000 to 12 000 years ago subsisted in part by collecting seeds of autogamous (self-pollinating) wild grasses (emmer and einkorn wheat, barley) and pulses (lentils, chickpeas, peas). However, fruits, nuts and other tree-related foods and fibres also formed an important part of the diet. Most of these woody species are highly heterozygous and do not come true-to-type from seed, which impeded rapid genetic improvement by seedling selection, thus prolonging domestication of these woody plants for thousands of years (Childe, 1958; Zohary and Spiegel-Roy, 1975; Janick, 2005; Janick, 2011).

Domestication of highly heterozygous plant species would depend on development of asexual propagation methods such as rooting of cuttings, layering or propagation by offshoots. According to Zohary and Spiegel-Roy (1975) modification and adoption by early agriculturists of these techniques in the third or fourth millennium allowed for domestication of fig, grape, pomegranate, and olive all of which root easily from cuttings and date palm, which was propagated by division of offshoots. Domestication of woody species only followed at approximately the beginning of the first millennium before Christ (BC) as these species do not root easily from cuttings. Domestication of woody species was thus due to the discovery of grafting and the concept of rootstocks and included, amongst others, fruit trees such as apples, pears and plums (Juniper and Maberly, 2006).

Exploring the contribution of the scion, rootstock and environment to the phenotype with regard to yield and quality in citrus trees would entail knowledge about the principles of grafting as well as the diverse nature of the *Citrus* genus pertaining to phenotypic expression and reaction to various environmental factors.

2.2 THE CONCEPT OF GRAFTING

Grafting can be defined as the natural or deliberate fusion of plant parts so that vascular continuity is established between them (Pina and Errea, 2005). In layman's terms this translates as connecting two or more pieces of living plant tissue together in such a way that they will unite and grow as one composite plant. The term scion refers to a piece of shoot or a bud that is cut, usually from a mature plant, to be inserted into the rootstock. The term rootstock refers to a plant which already has a healthy established root system onto which the scion will be grafted or budded and can either be clonally propagated via cuttings or other methods such as lair layering or tissue culture or in the case of nucellar embryony could be an immature seedling. An interstock is a section of stem inserted between a scion and rootstock, often used to overcome incompatibility.

A compound genetic system is created by uniting two (or more) distinct genetic genotypes through grafting or budding (Mudge *et al.,* 2009). For almost every area of plant growth and physiology, the control of scion traits by the rootstock has been widely documented (Koepke and Dhingra, 2013). This regulatory mechanisms has been investigated by Harada (2010) with regard to RNA molecule transfer between rootstocks and scions and it was also found by Kasai *et al*. (2011) that post-transcriptomal gene slicing of scion genes by the rootstock is possible. However, nobody has disputed the finding of Bailey (1928) that in a compound genetic system each of the genotypes maintains its own genetic identity throughout the life of the plant and should vegetative material of each part be tested, it will be genetically true to its origin.

An important matter that has to be kept in mind, when considering rootstocks for a certain scion, is the limits posed by compatibility. Compatibility is defined as the ability of two different plants grafted together to produce a successful union and continue to develop satisfactorily. Causes for graft failure other than genetic incompatibility that can either be due to adverse physiological responses between scion and rootstock and/or anatomical abnormalities of vascular system can be ascribed to anatomical mismatching, or in other words, poor artisanship and adverse environment factors (Kumar, 2011).

Plants in the grass family and other monocotyledonous plants cannot be grafted or budded, as they lack cambium and monocots cannot be grafted onto dicotyledonous plants. Conifers and other flowering plants, as well as many herbaceous and woody plants, can be grafted (Kumar, 2011). Gymnosperms are usually grafted scions whilst angiosperms are usually budded scions.

Rules for compatibility (Kumar, 2011) state that:

- The highest success in grafting or budding is achieved by grafting plants within or between clones
- Plants of the same genus and species can usually be grafted, even if it is a different cultivar or variety.
- Plants of the same genus but different species may or may not unite.
- Plants of different genera are less successfully grafted and plants of different families will not result in a successful graft.

Although, the actual when and where of the rootstock-scion concept is unknown, the principal use of grafting is known to be that of vegetative propagation, to assure that ramets (vegetative offspring) are genetically identical to the scion donor tree. Other useful attributes of grafting includes (Mudge *et al*., 2009):

- **Economical:** Genotypes or clones are grafted due to low success by other vegetative methods such as cuttings or layering.
- **Avoidance of juvenility:** Juvenility in woody plants can last several years in fruit trees to several decades in forest species. A cutting, taken from a mature tree maintains its flowering state, thus fruit producers can overcome the problem of juvenility by grafting a scion from a mature tree onto a rootstock. This rootstock can even be a seedling, as a mature scion grafted to a juvenile seedling will maintain its mature properties.
- **Cultivar change:** As new cultivars of various fruit trees are being bred and old cultivars go out of style, cultivar change can be speeded up by taking advantage of a mature root system. If the rootstocks are in a healthy state, new scions can be grafted on scaffold branches of an established tree that has been cut back to the rootstock, a process known as top-working.
- **Multiple cultivars:** A rootstock can also be grafted with more than one scion as a novelty. When self-incompatibility is a problem, as in cherry and apple, a polliniser can be grafted to achieve cross-pollination within a single tree.
- **Repair:** Various factors, physical or pathogenic, can cause bark damage (girdling) which adversely affects an established tree. Grafting techniques such as

inarching seedlings around the base of the injured tree can effectively save the tree. Other grafting techniques such as bridge grafting and brace grafting can also be used to repair a girdled stem or to strengthen trees by internal grafts between branches.

- **Size control:** Commercial farming practices and the need for profitability, demands the control of tree size. Although rootstocks can be used for invigoration of the scion cultivar, it is mostly dwarfing attributes that are needed commercially. Certain rootstocks will result in dwarfing or invigoration of the scion cultivar. In apple, a single scion cultivar grafted onto various rootstocks can result in trees ranging from 2 m to 10 m in height. In other species, certain interspecific scion/stock combinations will result in dwarfing, such as pear on quince and orange (*Citrus sinensis*) on trifoliate orange (*Poncirus trifoliata*).
- **Biotic and abiotic stress resistance:** The root system and the shoot system of a plant exist in different environments. Each has a different role in plant development and each makes a different contribution to agricultural productivity. Given the long generation time of trees (years), it could take a very long time, using standard plant breeding methods, to breed a tree to genetically optimize both root and shoot systems. Grafting on the other hand, has allowed agriculturists to mix and match different genotypes in the root and shoot systems, resulting in a genetically compound plant that performs better overall than either genotype alone. Just as rootstocks have been selected for controlling size of the scion, rootstocks have also been selected for resistance to various diseases, pests, and abiotic stresses.
- **Transfer of infectious agents:** Since all viruses are graft transmissible, cross protection through pre-immunizing with a mild strain virus is a principle whereby a mild strain added to a rootstock provides protection to the scion against the more virulent strain of that virus. Grafting is also used to transfer a phytoplasma (cell wall-less bacterium) to modify the growth habit of poinsettia, which induces a desirable branching (compact) growth habit. Similarly, the presence of latent viruses in certain apple rootstocks may actually improve performance of scions grafted onto those rootstocks, compared to virus-free clones. For example, apple trees on virus-free EMLA 9 rootstocks are usually more vigorous and less precocious and productive than the same scions grafted onto other M9 clones, such as M9-337, in which latent viruses have not been eliminated (Autio *et al*., 2001).

- **Physiological studies:** Grafting has been widely used in genetic and physiological studies to determine the transfer of mobile elements in plants:
  - **Genetic:** According to Liu *et al*. (2010) grafting allows exchanges of both RNA and DNA molecules between the grafting partners, thus providing a molecular basis for grafting-induced genetic variation. Apart from DNA-based plant viruses, there is no current evidence that would support movement of genomic DNA through the vascular system of a grafted plant. However, movement of plastid DNA across cellular barriers immediately adjacent to the graft junction has been demonstrated (Stegeman and Bock, 2009). It is also becoming evident that certain transcription factors, mRNAs, regulatory micro RNAs (miRNAs), small interfering RNAs (siRNAs), peptides, and proteins are mobile in the plant vascular system and thus, may cross the graft union. It has also been observed that when pPGIP-expressing transgenic plants are used as rootstocks onto which non-expressing scions are grafted, the pPGIP protein, but not the pPGIP-encoding nucleic acids, are exported to the scion, crossing the graft union via the xylem system (Aguero *et al*., 2005).
  - **Physiological:** This includes translocality of alkaloids and secondary metabolites (Nisar, 2012), transfer of the flowering stimulus (florigen) (Zeevaart, 2006) and transfer of growth substances such as cytokinin from roots to shoots (Kudo *et al*., 2010).

2.3 THE CONCEPT OF ROOTSTOCKS

Benefits of grafting infer the concept of rootstocks as well as the concept of specific rootstock effects and non-specific rootstock effects. Specific rootstock/interstock benefits are advantages gained by grafting that are due to the specific genotype of the rootstock or inter-stock and non-specific rootstock benefits would be grafting to achieve an objective that could be achieved by any compatible rootstock, regardless of its genotype (Mudge *et al.*, 2009). With regard to the above attributes of grafting, the following would pertain to non-specific rootstock benefits such as avoidance of juvenility, cultivar change, multiple cultivars, creation of unusual growth forms and repair. Specific rootstock (or interstock) benefits thus refer to the control of tree size, effects of rootstock on precocity (early flowering), biotic and abiotic stress resistance, transfer of infectious agents and physiological studies. (Mudge *et al.*, 2009). However, irrespective of the specificity of rootstock effect, there will always be an interaction between the scion and rootstock that can be either negative or positive or in the case of an interstock, the interaction will be three way in nature.

2.4 THE GENOTYPE: CITRUS DIVERSITY

Citrus belongs to the family Rutacaeae and sub-family Aurantioidae (Nicolosi, 2007). The crop is global with production in over 100 countries on six continents. Furthermore, citrus is the most important tree fruit crop in the world, with current world production far exceeding that of all deciduous tree fruits (such as apple, pears, peaches and plums). The area planted to citrus was estimated at two million hectares by the year 2000 (Saunt, 2000). Citrus is grown primarily between the latitudes 40° N to 40° S (Davies and Albrigo, 1994). The majority of commercial citrus production, however, is restricted to two narrower belts in the sub-tropics, roughly between 20 and 40° N and S of the equator (Castle, 1987; Saunt, 2000). Most citrus orchards worldwide consist of budded trees that combine favourable attributes of scions and rootstocks through grafting (Davies and Albrigo, 1994).

2.4.1 Origin and distribution of citrus

According to Nicolosi (2007) the oldest Chinese reference to citrus fruit appears in the book “Tribute of Yu” which pertains to a period between 2205-2197 BC. Much confusion exists regarding classification of the genus Citrus, and this confusion is not likely to be resolved soon. As more taxonomic research is conducted, gaps in our knowledge grow narrower or can become even more confusing when conventional wisdom is being challenged, such as has happened recently with molecular studies (Nicolosi, 2007). However, conventional wisdom holds that citrus and its related genera originated in south-east Asia.

2.4.2 Botanical classification of citrus

Several authors (Swingle, 1948; Hodgson, 1967; Swingle and Reece, 1967; Webber et al., 1967; Bijzet, 2006a; Nicolosi, 2007) have given detailed discussions of taxonomy and taxonomic groups in citrus. Although citrus is one of the major fruit crops in the world, there is a great deal of confusion in general citrus taxonomy. Regardless of the chaos, citrus seems definitely to belong to the subfamily Aurantioideae in the family Rutaceae.

Aurantioideae is divided into two tribes i.e. Clauseneae with five genera and Citreae with 28 genera. The subtribe Citrinae is divided into three subtribal groups namely: primitive citrus (five genera), near-citrus (two genera) and true-citrus (six genera). The genus Citrus belongs to the subtribal group true-citrus (Nicolosi, 2007). Taxonomy is not yet precisely established for the genus Citrus as taxonomic relationships among members of this genus were established by various scientists of whom Swingle and Reece (1967) and Tanaka (1954) were the most prominent. Unfortunately, these classifications differ considerably in number of species as Tanaka (1954) recognised 163 species but Swingle and Reece in 1967 only honoured 16 species. Most researchers prefer to use the Swingle system, represented in Figure 2.1. However, this is sometimes expanded to include some of Tanaka's species as the Swingle system does not provide a complete description of citrus systematics (Nicolosi, 2007). Relationships within this group of "true citrus" is important to citrus breeders as commercial citrus scions and rootstocks belongs to this group.

Currently, three ancestral species *C. medica* (L.), *C. grandis* (L.) Osbeck and *C. reticulata* Blanco are recognised in the sub genus *Citrus (historically Eucitrus)* (Barrett and Rhodes, 1976; Handa *et al.*, 1986; Nicolosi, 2007). The rest of the species in the genus Citrus have probably arisen by hybridisation among these ancestral species amongst themselves or with other genera from the sub genus *Citrus* such as *Poncirus* Raf., *Fortunella* Swingle or *Microcitrus* Swingle (Table 2.1). Well known hybrids such as oranges (*Citrus sinensis*) have become "convenience species" (Scora, 1988; Nicolosi, 2007).

Table 2.1 True or ancestral citrus vs. species of convenience (Bijzet, 2006a)

Type	Designation	Hybrid origin
Ancestral citrus spp.	*C. reticulata* *C. medica* *C. grandis*	Mandarin Citron Pummelo
Ancient hybrid citrus	*C. sinenesis* *C. aurantium* *C. limon* *C. aurantifolia* (Mexican lime, Acid lime etc.)	Pummelo x mandarin Pummelo x mandarin Mexican lime x citron (Pummelo x citron) x Microcitrus?
Modern hybrid citrus or species of convenience	Grapefruit Tangelo Tangor Lemonage Lemonimes Lemandarin, *C. limonia* (Rangpur lime) *C. latifolia* (Tahiti, Persian, Bearss lime) *C. volckameriana* Citrange Troyer C-35 Citrumelo Swingle	Pummelo x sweet orange Mandarin x grapefruit Mandarin x sweet orange Lemon x sweet orange Lemon x Mexican lime Lemon x Mandarina Mexican lime x citron or Mexican lime x lemon Lemon x sour orange Sweet orange x *Poncirus trifoliate* *P. trifoliate x* Washington navel *P. trifoliate x* Ruby blood orange Grapefruit x *Poncirus trifoliate* *P. trifoliate x* Duncan grapefruit

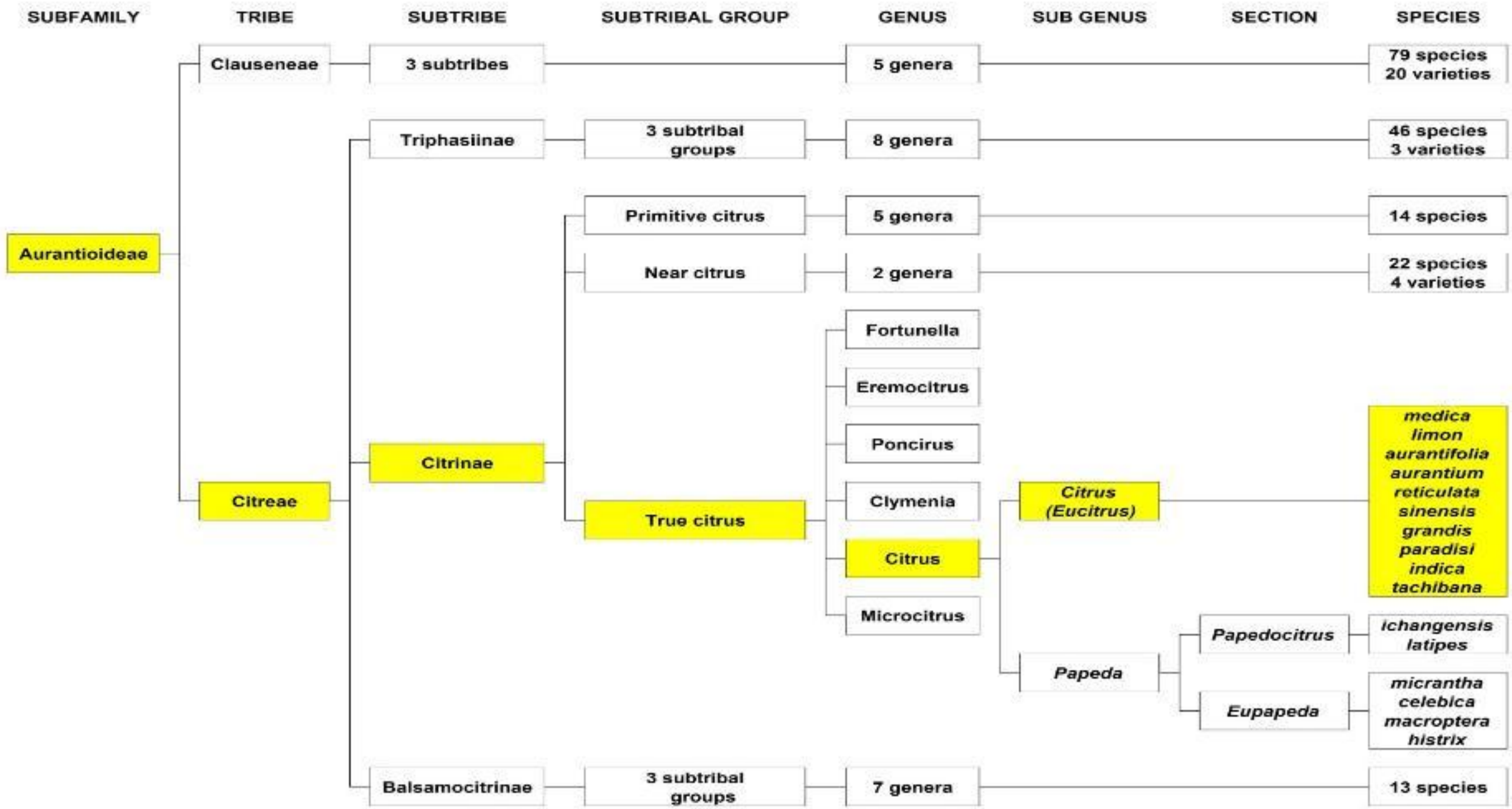

Figure 2.1 Placement of the genus *Citrus* in the sub-family Aurantioideae compiled from Swingle and Reece (1967)

2.5 THE ENVIRONMENT: CLIMATIC REQUIREMENTS OF CITRUS

Of the countless factors that must be taken into account when farming with citrus, climate is primarily the determining factor affecting citrus production with regard to both yield and quality. Due to it being impervious to human intervention, climate is the paramount factor influencing type and quality of citrus that can be grown successfully in certain areas, especially when aiming for export. Climate usually refers to temperature, day length, solar radiation (light), rainfall, humidity, wind, and atmospheric pressure. Within the areas of the world in which citrus are grown, temperature appears to be the main climatic factor that influences fruit (Luo, 2011). Zhang et al. (1992) reported a positive correlation between the growth rate for citrus fruit and temperature, rainfall and the duration of sunshine, while being negatively correlated to evaporation.

2.5.1 Temperature

Various crucial temperatures are applicable to citrus. Citrus trees originated in tropical and subtropical areas and are therefore not frost tolerant and are thus in South Africa curbed to areas with mild and almost frost free winters where temperatures almost never drop below -2°C. A minimum temperature of 2°C (especially in the absence of frost protection) and a maximum temperature of 35°C were identified as the temperature thresholds for citrus across its growing season (Luo, 2011). Rosenzweig *et al*. (1996) reported that a maximum temperature higher than 38°C may cause losses in citrus fruit set near the end of bloom and at 48°C will cause a 50% loss in fruit set. According to Luo (2011) sunburn and fruit losses occurs at temperatures of 40°C and higher. A drop in temperature to below 13°C initiates a dormant state in the tree as it was reported that the dormancy stage has an optimum temperature range of -4°C to 14°C (Luo, 2011). Optimal temperature ranges for flowering and fruit set were respectively 10-27°C and 22-27°C, while fruit growth was best between 20-33°C. With regard to fruit quality and maturation optimal temperatures for the development of soluble sugars was 13-27°C while rind colour development occurred between 8-48°C (Luo, 2011) The threshold for root activity is a soil temperature of 15°C. Maximum shoot growth occurs when temperatures reach between about 25 and 31°C and growth is slower at about 32 to 33°C (Pittaway, 2002).

Temperature relates to heat and heat summation relates to the energy available to the trees for all its physiological and growth processes. Heat units are used to obtain the total effect of maximum and minimum temperature (Khurshid and Hutton, 2005). Heat units are thus an index of daily day degrees above a base temperature (BT), which in the case of citrus is 13°C.

Daily heat units =AT- BT where AT is the average daily temperature and BT is the temperature under which no growth occurs.:

$$\text{HU for a day} = [(\text{Minimum} + \text{Maximum Temperature}) \div 2] - 13$$

This index can now be used to determine the total heat accumulated in a specific area during specific periods of development. It has been found that heat units are strongly correlated to growth rate and fruit quality, providing that there are no other serious limitations.

According to Ladaniya (2008) the minimum heat unit range (1000-1400) results in a poor growth rate while very high heat units of lowland tropics (HU =5000-6000) lead to faster growth but produce poor-quality fruits. Lower heat units delay growth and result in higher acids and lower sugar content. Heat units of different areas can be compared to known criteria for the different citrus types to determine the climatically suitability of an area. Figure 2.2 shows the heat unit criteria for the commercial citrus types.

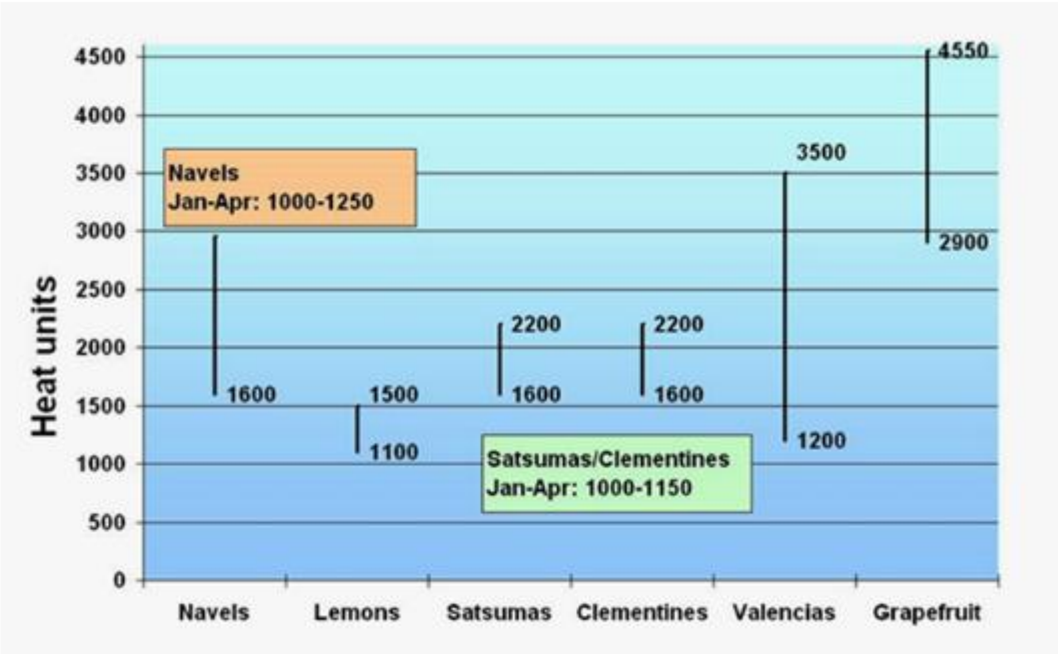

Figure 2.2 The heat unit criteria for a few main citrus types (Bijzet, 2006b)

However, the heat unit concept:

- does not reflect detrimental high and low temperatures at which harm to the tree can occur
- does not take other factors such as water stress into account
- assumes all cultivars to have a minimum growth rate of 13°C and
- assumes the fruit growth curve to be a straight line instead of a sigmoid

2.5.2 Day length and light

Most woody tropical plants are affected by day length and citrus is no exception. Growth is positively correlated with day length. In South Africa sunlight hours do not seem to be a limiting factor in most parts of the country except in certain "mistbelt" climates where the temperatures are reduced due to low light intensities culminating in less carbon dioxide assimilation with a consequent influence on fruit growth and quality. Conversely, flowering in citrus has been found not to be sensitive to day length but dependant on temperature and water stress. Optimum sunlight inception by the total leaf area of the citrus tree can be facilitated by the correct row direction, planting distances and manipulation such as pruning (Iglesias *et al*., 2007).

2.5.3 Rainfall and humidity

Water availability is of utmost importance to the adaptability of cultivars to a certain environment. Seasonal rainfall patterns in South Africa are erratic and do not correlate well with the water requirements of commercial citrus trees. In winter rainfall areas, rainfall occurs during the fruit maturity and resting phases whilst in summer rainfall areas precipitation occurs too late for fruit set. In South Africa, commercial citrus plantings are irrigated and dry land planting is thus not encouraged. Humidity is a determining factor in morphological and pathological disorders such as sheepnose and Altenaria infections. Excessive soil water can cause Phytophthora root rot.

2.5.4 Soil attributes and soil climate

Citrus trees need deep soil with a pH of between 6.0 and 6.5 as well as good surface and internal drainage. For optimum water provision, ideal citrus soil will have no mottled or structured layers within 1 m of the soil surface, be red, yellow-brown or brown in colour with clay content of 10 to 40%.

A less familiar concept is the climate of the soil. The root environment of a plant i.e. soil temperature, soil water and even nutrition, is less subject to significant and rapid transformations than plant parts exposed to the atmosphere. However, soil climate is important since the uptake of water and nutrients is regulated by soil temperature. At a depth of 30 cm soil temperatures can range from 6 to 16°C in winter and between 24 and 30°C in summer. Temperatures below 15°C restrict the ability of roots to absorb water, whilst the assimilation of nitrogen is best in warmer months.

2.5.5 Wind

An otherwise perfect citrus area can be rendered unsuitable due to a high frequency of wind at the wrong time or at high velocities. The harm that is done can be twofold in that hot winds can burn trees and cause die-back due to excessive moisture loss caused by transpiration and mechanical abrasions can cause cosmetic damage rendering fruit unsuitable for export.

2.5.6 Citrus producing areas in South Africa by geographic area

Citrus is produced in a belt spreading approximately 40° latitude on each side of the equator in the tropical and subtropical areas where soil and climatic conditions are favourable. However, the majority of commercial production is currently restricted to two subtropical bands more-or-less between 20° to 40° north and south of the equator. In South Africa, citrus producing regions are characterised by geographical, topographical and thus climatic diversity and range of latitudes 17° to 34°S (Bijzet, 2006b).

South Africa has a vast number of different climatic regions. The classification by Barry (1996) of these regions into a few major zones is still in use and is given in Table 2.2. The coastal regions of Southern Africa can be regarded as frost-free. The Lowveld and northern parts of Mpumalanga can also be regarded as frost-rare to frost-free. Although not perceived as an arid country, a large portion of Southern African land area receives less than 500 mm rain per annum making it relatively arid. The western and southern Cape regions have a Mediterranean-type climate with winter rainfall, whereas the northern and eastern regions are all summer rainfall areas with a more semi-tropical to subtropical climate. Table 2.2 summarises the climate zones that are suitable for citrus production and the type of citrus suitable for the specific climate zone.

Table 2.2 Climate zones suitable for citrus production and type of citrus per zone (Barrry, 1996)

Climate zones	Climate description	Citrus suitability	Best suited for:
Hot production areas: Thipise, Letsitele Letaba, Hoedspruit Swaziland Lowveld Malalane Pongola Nkwalini	Hot humid (<300 m elevation) Hot dry (300 to 600 m elevation)	This area is suitable for the production of high quality grapefruit and Valencia oranges, and to a lesser extent midseason oranges and certain mandarin types such as Minneola tangelo. A small amount of lemons, Tahiti limes and pummelos are also produced in this region.	Grapefruit Valencia Lemons Limes Pummelo
Intermediate: Marble Hall, Nelspruit, Karino, Barberton, White River, Hazyview, Leataba, Levubu	It falls between the hot, low-lying areas and cool, high-lying areas, i.e. between 600 and 900 m elevation,	These areas are suitable for the production of Valencia and midseason oranges and lemons, and are marginally suitable for grapefruit (too cool) and Navel oranges (too warm).	Valencia Midseason Lemons
The cool, inland production region: Rustenburg Lydenburg Potgietersus Zebediela	High-lying (above 900 m elevation	Suitable for the production of Navel oranges and lemons, the warmer microclimates are suited to Valencia oranges, and to a lesser extent certain mandarins, such as Clementine, Nova and possibly Temple tangor.	Navels Valencia Lemons Clementines
The cold production region: Midlands of the Eastern Cape Sundays River Valley Gamtoos Valley Western Cape Southern Natal	This is the semi-coastal areas situated in southern latitudes, between 32°30' and 34°30' S in the eastern, southern and western Cape.	High quality Navel oranges, Satsuma, Clementine and Nova mandarins, and lemons are produced in these areas, while the warmer microclimates in these areas are suitable for Valencia orange production.	Navels Valencia Satsuma Clementines Mandarins
The semi-desert region: Vaalharts Orange River basin	This zone is characterised by extremes: hot summers and cold winters with the occurrence of frost.	In the cooler Vaalharts area, Navel and Valencia oranges are produced, whereas grapefruit and Valencia oranges are produced in the hotter lower Orange River area.	Navel Valencia

2.6 THE IMPACT OF ENVIRONMENT ON GENOTYPE PERFORMANCE

Citrus cultivar performance is twofold. The consumer is mainly concerned with fruit quality and only indirectly with fruit production as the profitability of production has a bearing on the price they have to pay for the produce. The producer, on the other hand, is more concerned with the profitability of producing the fruit than with the quality *per se*. However, quality is also of major importance to the producer due to the pressure associated with global trade as well as the competitive international market with consumers demanding high quality healthy fruit. The genotypes (scion and rootstocks) involved as well as the environment (climate) influence both production and quality and there is a considerable diversity in this regard amongst citrus genotypes in their response especially with regard to quality (Zekri, 2011).

2.6.1 Production as influenced by climate

Yield relates to tree size, flowering, fruit set and fruit drop which are influenced by major climatic aspects such as day length, solar radiation (light), rainfall or available water, humidity and wind. High productivity is the effect of three critical stages during fruiting namely flowerbud differentiation, fruit set, and fruit enlargement (Goldschmidt, 1999). In explaining the differences with regard to horticultural characteristics in different commercial citrus areas, temperature is regarded the most important factor (Spiegel-Roy and Goldschmidt, 1996; Anonymous, 1997; Bijzet 2006c). Low temperatures are ideal, but not essential for flowering. Water stress can have the same result as low temperatures with regard to flowering. The intensity and duration of water stress has a direct bearing on the intensity of flowering that occurs. The dormancy in winter rainfall areas are cold induced whilst in summer rainfall it is drought induced.

Fruit set and fruit drop influences yield and are dependent on the cultivar and environment. Moisture stress and temperature are amongst the most important factors affecting fruit set and drop. High temperatures and severe moisture stress in the plant tissue not only cause excessive blossoming but also fruit drop and thus lower yields. Root temperature does not influence floral induction (Anonymous, 1997).

2.6.2 Influence of climate on fruit quality

Quality is a complex perception of many attributes that are simultaneously evaluated either objectively or subjectively. There are many definitions and standards set by producers, researchers, and consumers. However, at the first level, fruit quality is simply the sum of those attributes that create and enhance consumer appeal and, in citrus, pertains to size, colour, taste (sweetness, flavour and texture), rag toughness and keeping quality. Fruit quality is thus very important in the production of export fruit.

Fruit size: According to Guardiola and Garcia-Luis (2000), the importance of fruit size as a parameter of citrus fruit quality has grown markedly in recent times. This can be attributed to consumer preference of larger fruit. Fruit size has thus become as important as yield in determining the profitability of citrus production.

Fruit size is primarily regulated by the number of competing flowers and fruitlets (crop load), by temperatures, particularly during early development, by available soil moisture through most of the fruit development period and by choice of rootstock. Although a high correlation between fruit growth and air temperature exists, canopy leaf area to fruit numbers is probably also a factor to consider. All of these factors have a larger influence earlier than later in fruit development. Fruitlet growth results from the accumulation of dry matter and water which is determined by the sink capacity of the fruitlet and the availability of water and nutrients (Guardiola and Garcia-Luis, 2000).

There are three stages of fruit development which, based on Valencia in South Africa, starts in September with a slow growth but intense cell division period lasting approximately 9 weeks (Stage 1) after which the fruit size is about 20 mm in diameter. During Stage 2 from November the initial slow growth changes to a rapid growth due to cell enlargement. This stage lasts between 28 to 30 weeks and is regarded as the most critical period with regard to fruit development and should be supported by optimum heat, soil moisture and control of excessive winds. Stage 2 is followed by approximately 11 weeks (Stage 3) in which the fruit reach horticultural maturity. This stage is typified by change in peel colour, decrease in acidity and increase of total soluble solids in the juice. However virtually no fruits growth takes place during this stage (Bijzet, 2006a)

Ideal temperatures for enhancement of fruit growth rate appear to be in the 20 to 25ºC range (Bijzet, 2006c), with both lower and higher temperatures reducing growth rate. However, it was found that in warmer, more humid climates, larger fruit size can be obtained even with a large crop due to higher temperatures from bloom through the first half of fruit development that increase the rate of fruit growth (Reuther and Ríos-Castaño, 1969). Higher temperatures during the first stage of fruit growth, in the cooler spring climates of Mediterranean areas, can also significantly increase fruit size (Marsh *et al.,* 1999).

Optimal soil moisture availability during the initial fruit development stages will facilitate fruit growth, whilst heavy rains or irrigation later in fruit development could also lead to larger fruit (Anonymous,1997).

External fruit quality: External fruit quality pertains to the rind attributes such as thickness and colour. Rind thickness is largely determined by temperature, humidity and water supply during stages two and three of fruit development. Yamanishi and Hasegawa (1995) found the rind of pummelo to be thinner in shaded trees, while fruit grown in humid areas has thinner rinds than those grown in desert areas. Rind thickness affects internal quality, as it is one of the many factors said to have an influence on total soluble solids (TSS) of citrus and the amount of juice in the fruit (Anonymous, 1997). Rouse and Zekri (2006) states that temperature regimes during fruit growth and development generally play a dominant role in influencing fruit morphology as can be seen from Table 2.3.

Table 2.3 Influence of climate on external quality (Anonymous, 1997)

Attribute	Effect
Peel texture	Peel (rind) is smoother under humid than arid conditions. The oil glands tend to be less pronounced as well.
Peel thickness	Severe drought causes fruit to have thicker peels. This can clearly be seen on grapefruit following a very dry summer in a summer rainfall area.
Shape	Flatter fruit is produced in cool humid climates than in hot arid climates. Satsuma mandarins grown in warmer, drier regions in the north usually have necks and are larger and more round than the same cultivar grown in the cooler more humid citrus regions of the Cape.
Peel colour	Colour is a function of the breakdown of chlorophyll to reveal the yellow and red carotenoids in the peel. In the scenario of high minimum temperatures, fruit stays green and has to be chemically degreened.

Creasing, also known as albedo break-down, is a pre-harvest disorder that affects the surface of the fruit, rendering it unacceptable to the market. This rind disorder was first reported in 1938 in South Africa (Le Roux and Crous, 1938) and can according to Gilfillan *et al*. (1980) cause significant individual orchard losses often exceeding 50% in Navels and Valencias. According to Joubert and Joubert (1957) and Holtzhausen (1981) the variation in the incidence and severity of creasing from year to year is attributed to climatic differences. Gambetta *et al*. (2000) could not establish a relationship between temperature and the incidence of creasing in the early stages of fruit development, as was reported by Jones *et al.* (1967) in a southern hemisphere study on 'Washington' navel oranges. Instead, Gambetta *et al*. (2000) did find that high mean relative humidity from full bloom until physiological fruit drop was related to a higher incidence of creasing. However, a positive correlation between creasing and the average maximum and minimum temperature range, prior to flowering, was observed in the Northern Hemisphere by Ali *et al.* (2000).

Internal quality: Four factors are normally mentioned when referring to internal quality of citrus fruit (Anonymous, 1997). These are juice percentage, TSS, titratable acid (TA) content and the TSS/TA ratio. Other internal characteristics important with regard to marketing are colour, maturity date (early or late) and seed content as well as rag strength in oranges and grapefruits. Development of internal pigmentation can be attributed to either anthocyanin or lycopene depending on the citrus type (Table 2.4).

Table 2.4 Influence of climate on internal fruit pigmentation

Citrus type	Climate effect on internal pigmentation	Comment
Blood oranges	Blood oranges need cooler conditions during stage 3 for heavy pigmentation to take place. Fruit from very hot, arid areas sometimes does not display any pigmentation.	Colour is attributed to anthocyanin
Grapefruit and Pummelos	Lycopene concentration is definitely higher in hot climates than in cooler climates. Very hot climates (above 35°C) on the other hand can inhibit lycopene accumulation.	Colour attribution is through lycopene development during stage 2 and 3.

Maturity: Citrus is a non-climacteric fruit, meaning they ripen without ethylene and respiration bursts and will thus not ripen any further once harvested (Spiegel-Rroy and Goldschmidt, 1996). Maturity advances very rapidly in tropical climates whereas fruit in cooler areas are later maturing and are harvested over a longer period. Maturity standards are expressed as a ratio of sugars, expressed as total soluble solids (TSS) to titratable acid (TA). The acid content of the fruit referred to, is citric acid and it is highest early in the season and decreases as fruit matures (Monselise, 1986). Fruit acidity is determined by a procedure known as titration.

Total Soluble Solids (TSS): Total soluble solids (TSS) or degrees Brix (ºBrix) refers to the total amount of soluble constituents of the juice. These are mainly sugars, with smaller amounts of organic acids, vitamins, proteins, free amino acids, essential oils and glucosides. Approximately 85% of the total soluble solids of citrus fruit are sugars - so TSS is a guide to the sugar content of fruit. Fruit sugar levels generally increase as the fruit matures. However, levels can decrease when fruit are over-matured (Hardy and Sanderson, 2010). No clear-cut relationship could be established between temperature and TSS. However, as the TA decreases TSS and juice content increases. TSS levels are high in intermediate sub-tropical climates but TSS levels tend to be low in hot tropical climates and very cool subtropical climates. However, sunlight hours seem to be important with high levels of TSS where light intensity is the greatest. Controlled irrigation (moisture stress) can help to raise TSS concentrations in areas where the climate causes competition between fruit and vegetative growth. TSS levels in grapefruit and pummelo cultivars in contrast to oranges and mandarins do not differ significantly between tropical and subtropical climates (Anonymous, 1997).

Titratable acidity (TA): Acid decreases with an increase of temperature except for lemons and limes. Titratable acid (TA) levels are higher in semiarid or arid subtropical and coastal climates (Zekri, 2009). At higher temperatures, the acid content is inclined to decrease at a faster rate, rendering it low at harvest time. The chemical changes in the juice of acid citrus, like lemon, however, are different from those in the juice of ‘table’ citrus. Organic acid content in citrus juice increases up to maturity (Widodo *et al*., 1996). Acid levels can drop due to high amounts of water, either irrigated or rainwater. A decrease in acid content is associated with an increase in TSS and juice quality (Anonymous, 1997). Virtually no fruit growth takes place during this stage.

Ratio of TSS to acidity: With maturity, the pH of the juice increases as the acid decreases relative to the TSS. A specific ratio indicates maturity (ripeness/edible quality) thus the TSS/TA ratio is the most widely used criterion of maturity and for determining harvest date in citrus. According to Yamanishi and Hasegawa (1995) oranges of good quality have a 8:1 minimum ratio, while the minimum standard for grapefruit maturation is considered 5.5:1 to 7.2:1 while for pummelo a ratio in the range of 8:1 to 10:1 can be considered to indicate good quality. If fruit is allowed to mature further, the acid content continues to decrease and the ratio increases and the fruit becomes overripe and tasteless (Anonymous, 1997). The practical use of the °Brix-to-acid ratio in determining maturity is based on the linear function between these two determinants (Widodo *et al*.,1996). Heavy or continuous rainfall during harvesting time e.g. in the Eastern and Western Cape can cause acid levels to drop even lower resulting in a higher ratio and thus insipid fruit that are not acceptable for the export market.

General effects of warmer climates on citrus fruit development are as follows: regarding internal quality, high temperatures accelerate fruit growth, the fruit has less time to accumulate soluble solids, and the high respiration rate leads to use of carbohydrates in respiration, which further reduces available sugars for accumulation in the fruit. The high respiration rate may lead to faster turnover of acids (Purvis, 1983) with resulting rapid dissipation of acidity level at higher temperatures (Reuther, 1973). Year to year variation in weather in a given climatic zone can lead to significant fruit quality variation (Albrigo, 1993).

Fruit toughness (rag strength): Toughness or rag is the internal texture of the fruit as perceived when chewing the segments. This attribute is influenced by climate, as fruit from hotter and dryer regions tend to have a stronger fibre and thus a higher toughness than fruit from cooler areas (Rabe *et al*., 1987).

2.6.3 Rootstocks

It has long been recognised that rootstocks influence citrus yield, fruit quality and tolerance to stress caused by biotic and abiotic factors of citrus fruits produced by the scion cultivar (Bitters, 1961; Gardner, 1969; Castle, 1987; Monteverde *et al*., 1988; Recupero *et al*., 1992; Sosa *et al*., 1992; Georgiou and Gregoriou, 1999; Georgiou, 2000; Barry and Castle, 2004). However, understanding the role of rootstocks in fruit quality is a complex task. According to Castle (1995) it is unclear how rootstocks exert their influence on fruit quality.

However, water relations, nutrition, and plant growth regulators are undoubtedly among the most important factors involved (Castle, 1995). Until about 1840 all citrus was grown as seedlings (Saunt, 2000). The search for and use of tolerant rootstocks became eminent when foot rot (*Phytophthora* spp.) manifested as a major disease. The second disease that shaped the history of rootstocks was citrus tristeza virus (CTV). Sour orange seedlings that were initially used as *Phytophthora*-tolerant rootstock are highly susceptible to CTV and in South Africa and Australia where it was exclusively used as rootstock, it devastated large parts of the industry (Castle, 2010; Saunt, 2000). This influence of the rootstock on the scion is believed to be more common and more profound in citrus than in other fruits (Cummins and Aldwinckle, 1983; Castle, 1987). According to Castle (1995) little rootstock effect has been demonstrated among deciduous crops as compared to citrus rootstocks, when fruit quality is measured as physical traits and chemical composition.

2.6.4 Fruit quality as affected by scions and rootstock

Fruit constituents as quality component: Rootstocks have been reported to affect external and internal fruit characteristics such as fruit size and weight, rind thickness, juice content, total soluble solids concentration, and total acids (Bitters, 1961; Wheaton *et al*., 1990). According to Gardner (1969) the physiological basis of this influence has been a matter of speculation but found with regression analysis that 40% of the variation could be attributed to rootstock, and the remainder to fruit size.

Gardner (1969) concluded that the leaves of the scion supplies carbohydrates to the fruit but the rootstock determines the amount. This was supported by Taylor and Dimsey (1993) who found that scion and/or rootstock significantly influenced leaf nutrient composition in orange and mandarin trees in all citrus field trials assessed. Smith (1975) found rootstock effects on the leaf nutrient composition of navel orange trees to be more widespread than scion effects, while for mandarins, the effects for rootstock and scion were equal. Citrus type and species must therefore be taken into account when analysing concentrations of nutrients in leaves.

Juice volume (%) as quality component: The volume of the juice is said to have an influence on the percentage of soluble solids. With an increased juice volume, a decrease in soluble solids was noted and higher juice content was correlated with the smaller size of the fruit (Barry and Castle, 2004). Valencia oranges with thicker rinds contained less juice than those with thinner rinds showing a negative correlation. Considerable variation in juice content has, however, been found from season to season, between areas, varieties, rootstocks, and cultural practices (Bitters, 1961; 1986).

Total soluble solids (TSS %) as quality component: The citrus industry prescribes a minimum TSS percentage as a quality feature. TSS content also forms the basis of payment for fruit by some juice processors in a number of countries, especially where the trade in juice is based on frozen concentrate. The lower the TSS content of fruit the lower the yield of concentrate produced from it. TSS is often expressed as kg $tonne^{-1}$ (Hardy and Sanderson, 2010). Research and experience have found that rootstocks in addition to climate, profoundly influence the TSS content (Barry and Castle, 2004). Barry and Castle (2004) gave evidence to support a hypothesis that drought stress can cause the accumulation of TSS through osmotic adjustments, which render a possible explanation for rootstock effects on TSS. According to Zekri (2009) maximum levels of TSS are usually attained in the mid-tropics and in humid subtropical regions with warm winters.

Titratable acidity: Rootstocks tend to affect the total acidity of citrus juice and there is a significant difference in acidity between varieties maturing at different seasons and various varieties maturing within a given season (Bitters, 1961). Low acidity is an important requisite in selecting early-maturing varieties and lemon- and lime rootstocks such as rough lemon and Palestine sweet lime are able to reduce acidity. Cultivars budded to trifoliate orange and its hybrids such as the citranges, citremons and citrumelos, tend to have increased levels of acidity in the fruit. Acidity can also be increased by using certain grapefruit stocks, tangelos such as Sampson tangelo and *Citrus icangensis* hybrids such as Yuzu (Bitters, 1961). Total acid (TA) levels are generally highest in semi-arid or arid subtropical and coastal climates and decline more slowly as fruit mature, compared with other climates (Zekri, 2011).

In summary it can be said that fast-growing, high yielding vigorous rootstocks such as rough lemon, Volkamer lemon and *Citrus macrophylla* are responsible for larger fruit with thicker, rougher peel and lower concentrations of TSS and acid in the juice. Mandarins on these rootstocks tend to bear fruit that are puffy, hold poorly on the tree and have a high incidence of granulation. The most preferred rootstocks for most citrus types are slower growing rootstocks such as trifoliate orange and trifoliate hybrids that do not produce vigorous vegetative growth, but rather produce small- to medium-size fruit with a smooth peel texture and high TSS and acid content in the juice.

2.7 STRUCTURE OF THE BREEDING PROGRAMME

The scion breeding process implemented by the ARC-ITSC since 1992 has been modified in 2002 and is depicted in Figure 2.3.

Breeding is dependent on a variety of good breeding parents. Specific combinations are made by means of hand pollination, during peak flowering time (August to October). During autumn to winter of the next year (Year 1), the resulting fruit is harvested and the seeds are germinated in the nursery. Seedlings are budded to rootstocks during the following year (Year 2) and manipulated in the nursery to reach a specific height after which it is transplanted into a trellising orchard (Year 3). Seedling trees are known to have a long juvenile period (5-10 years) but fruit can be expected from “Year 6” onward due to various manipulation techniques. Bearing trees are then evaluated for three consecutive years. Potential selections are immediately multiplied for better evaluation and the orchard is removed after 10 years, disregarding all trees that have not yet come into bearing. Various role players will be allowed to view the selections and put forth their proposal for commercialisation. Radiated material from the mutation-breeding programme will follow the same modus operandi.

With regard to rootstock breeding the current objective in the ARC-ITSC breeding programme is to develop disease resistant/tolerant rootstocks, which impart favourable horticultural characteristics to the main commercial cultivars. Rootstocks should be adapted to problematic soils (calcareous, sandy) and preferably control tree vigour. Although the breeding process is the same in essence as that for the scion, it is actually more complex as evaluation not only includes the same traits as the scion but also has an additional focus of having to be resistant/tolerant to various diseases. The resulting seedlings in Year 1 (Figure 2.3) thus have to be screened first for the various diseases.

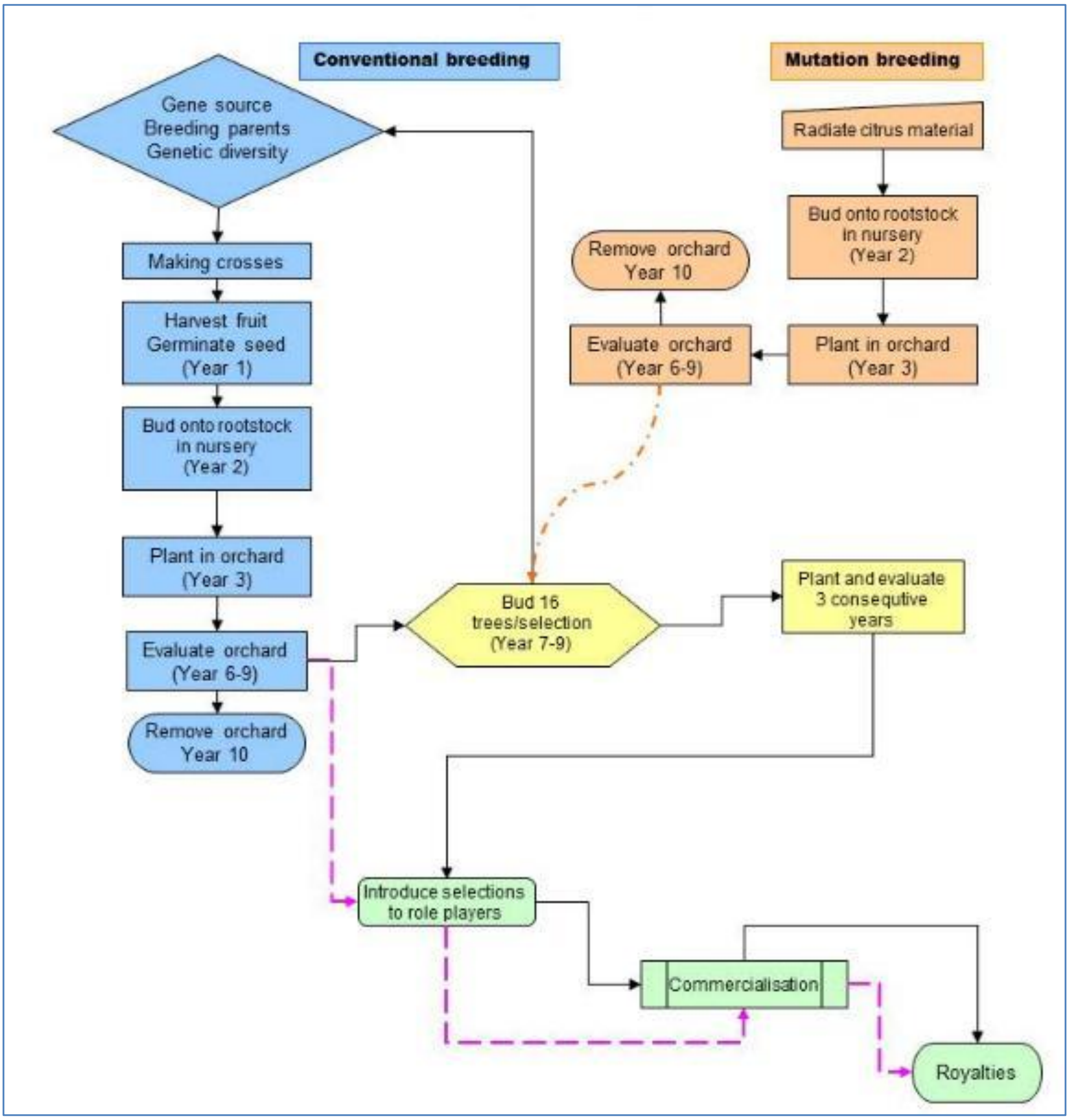

Figure 2.3 The scion breeding process implemented by the ARC-ITSC since 2002

2.1 STATISTICAL ANALYSIS METHODS OF GENOTYPE BY ENVIRONMENT INTERACTIONS (GEI)

2.1.1 Defining G, E and I

Researchers and breeders have to rely on the phenotype (visible traits) of a plant to infer its genetic potential. The phenotype refers to the physical appearance or visible traits of an individual and this may be observable at a physical, morphological, anatomical and physiological or biochemical level which changes continually depending on the interaction of the genotype with the environment (Falconer and Mackay, 1996). This is called the genotype by environment interaction or G x E interaction (GEI) where the G represents the genotype. The term genotype refers to the full complement of genes inherited by an individual that is important for the expression of a trait under investigation, and it is a fixed character, that remains constant and unchanged by environmental effects throughout the individual's life. With regard to the comparison of plant material, the term genotype refers to a cultivar (i.e. material that are genetically homogenous, such as pure lines or clones) rather than to an individual's genetic make-up (Annicchiarico, 2002).

According to Basford and Cooper (1998) the environment includes biophysical factors (water, soil fertility, temperature, disease), that influence the growth and development of a genotype. However, the environment is more than that and can rather be described as the sum total of the effects of physical, chemical and biological factors on an individual other than its genotype (Yan and Kang, 2003) . It is thus evident that GEI occurs in every aspect of biological science, and as a result, any scientific inference made from research is conditional because of the existence of GEI (Cooper and Byth, 1996).

In horticulture, GEI is perceived to be present when different cultivars or genotypes respond differently to diverse environments. To be able to detect and quantify GEI by means of statistical methods, measurements on at least two cultivars in at least two diverse environments is needed (Kang, 1997). The basic model representing GEI as per Falconer and Mackay (1996) is:

Phenotype (P) = Genotype (G) + Environment (E) + G & E interactions (GEI)

When GEI is present, the effects of genotypes and environments are statistically non-additive and the differences observed amongst the genotypes thus depend on the environment (Hühn, 1996). It is thus perceivable that GEI may lead to different rank orders of genotypes in different environments. Researchers agree that GEI is only of consequence if it causes a significant change in the ranking of the genotypes in different environments (Farshadfar *et al.*, 2012). This differential response of the cultivars to diverse interaction, is referred to as crossover interaction, and is depicted by intersecting lines on a graph. Non-crossover interactions represent changes in magnitude of (quantitative) genotype performance, but rank order of genotypes does not change. The result is that genotypes in a group of genotypes that are superior in one environment stay superior in the group in other environments.

Figure 2.4 illustrates a single trait (phenotype) of two genotypes (a, b) in two environments (x, y). For the illustrations the relative positions were assigned with the criterion, that genotype A in environment X always had the highest rank. The relations in 2.5a to 2.5d are considered as instances of no interactions while the others could be cases of significant interactions. Figure 2.4a constitutes neither genetic nor environmental differences. A trait could differ due to a genotype being superior but be unaffected by environmental changes (Figure 2.4b) or expression of a trait can be affected by the environment but be identical across the genotypes (Figure 2.4c). A trait can change across both the genotypes and the environments with the nature of the change being additive thus both genotype and environment enhances/decreases the appearance of the trait (Figure 2.4d).

When the phenotype manifest itself in a complicated way (lines diverge, converge or cross) it implies that there is not an additive genotypic effect (Grishkevich and Yani, 2013). The change could, for instance, be more profound in one environment than in the other as in Figure 2.4e (G>I>E). Alternatively, an environment might have opposing effects across genotypes (Figure 2.4f-h). The magnitude of the G, E and GEI in the equation P= G + E = GEI causes different scenarios.

The most important G by E effects for targeting cultivars or for selection of material are the crossover type affecting top-yielding genotypes. Such effects imply a change of ranks between environments rather than a simple variation in the extent of the difference between genotypes (Baker, 1988).

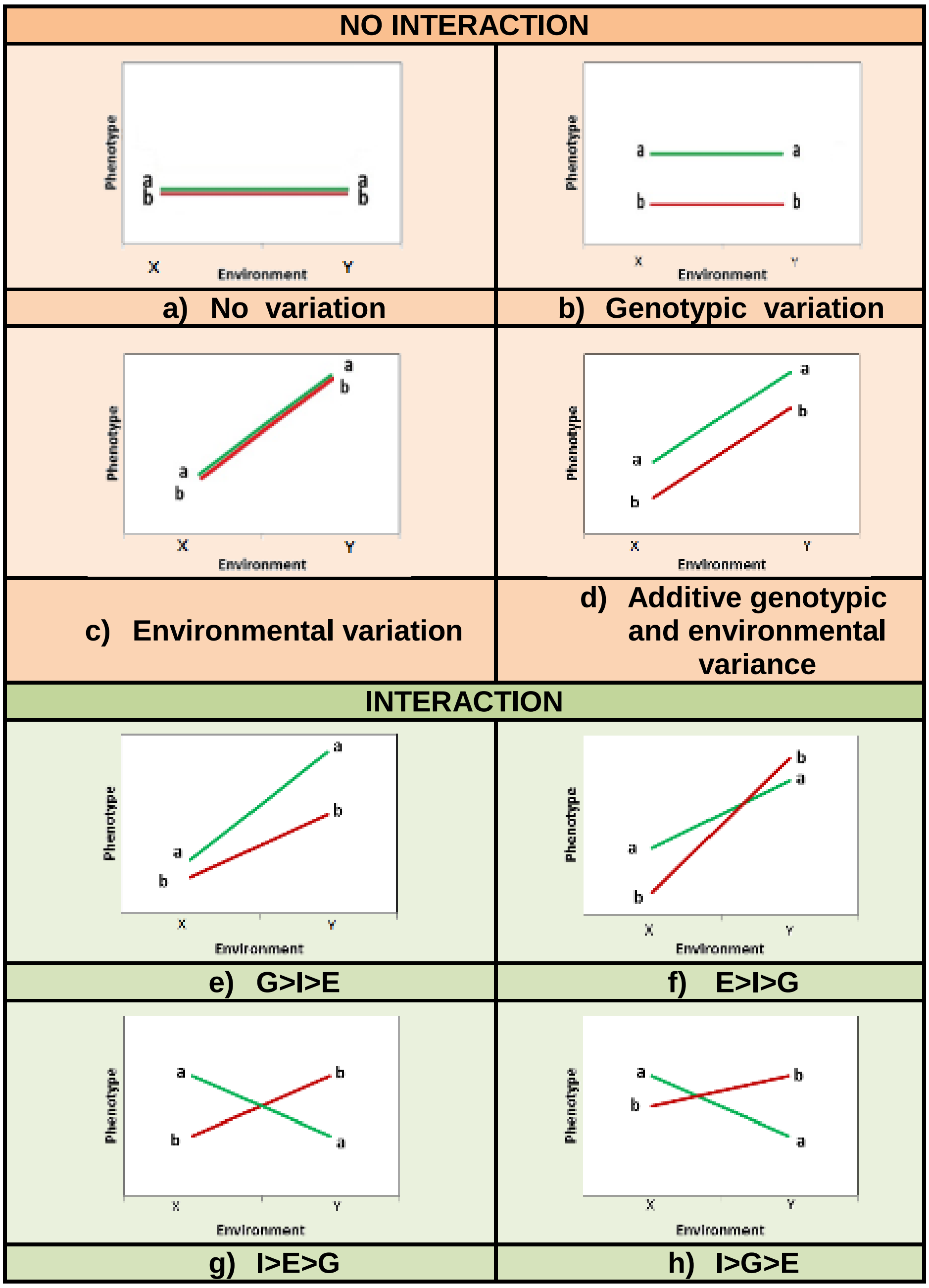

Figure 2.4 Modes of phenotypic variation across genotypes and environments

(A, B = genotypes; X, Y = environments; G, E, I = average effects of genotypes, environments and interactions, respectively). For the illustrations the relative positions were assigned with the criterion, that genotype a in environment X always had the highest rank)

GEI has been a focus of plant breeders as early as the 1950's. Numerous studies have shown that a proper understanding of the environmental and genetic factors causing the interaction as well as an assessment of their importance in the relevant G by E system could have a large impact on plant breeding (Magari and Kang, 1993; Basford and Cooper, 1998). These studies have mostly been in agronomy and animal breeding.

According to Kang and Gauch (1996) results from GEI can help to reduce cost of expensive genotype evaluation by identifying unnecessary testing sites and assisting with decision-making to fine-tune breeding programmes. The study of GEI is therefore particularly relevant to countries that have diverse agro-ecologies, as is the case in South Africa.

2.1.2 Multi-environment trials

The very diverse climatic conditions and soil types of South Africa as well as the diversity within citrus amplify the problem of GEI even further. When selecting genotypes in annual and some perennial crops, scientists circumvent this problem by comparing performance in yield trials over several environments and years to ensure that the selected genotypes have a high and stable performance over a wide range of environments (Breedt *et al*., 1996, Gmitter Jr *et al*., 2007, Castle *et al*., 2010). These are called Multi Environment Trials (METs) and often (not always) consists of the same set of cultivars planted in the same year at different localities (Gauch, 1988, 1992). Although promising selections of tree crops are usually also evaluated at different localities, the aim is more to test the future performance of these selections per locality. Multi-environment trials (METs) are routinely conducted in major cropping industries throughout the world to gain insights into GEI and to identify superior cultivars suited to particular environments that are high yielding, stable and more adapted to regional climatic conditions. METs also define the target population of environments (TPEs) and subdivision for further selection and evaluation. The purpose of a MET is thus to predict the performance of new cultivars (yield and quality) relative to a standard cultivar in different climatic zones and crop years. Investigations of METs are thus a prerequisite for any meaningful cultivar evaluation and recommendation procedures (Yan and Hunt, 2001).

2.1.3 Strategies for coping with GEI

According to Eiseman *et al*. (1990) GEI can be dealt with in three ways namely: (1) ignore the phenomenon by using genotypic means across environments even when GEI exists; (2) avoid GEI; or (3) exploit GEI in breeding objectives.

Strategies for exploiting G by E can be based on either broad or on specific adaptation (Lin *et al*., 1986).

- **Broad adaptation** is the consistent performance of a cultivar across a range of environments (high mean across environments).
  - It will not necessarily identify the best genotype for a specific environment
- **Specific adaptation** is the subdivision of environments into smaller regions so that there is little GEI within each small region. Cultivars are then selected and recommended per sub-environment.
  - A separate breeding programme will have to implemented in each of the sub-regions, minimising the resources available.
- Evaluate a common set of breeding material across environments but make specific recommendations for each environment.

2.1.4 Concepts of plasticity and stability

Phenotypic plasticity is the ability of an organism to change its phenotype in response to changes in the environment. The term was originally used to describe developmental effects on morphological characters, but is now more broadly used to describe all phenotypic responses to environmental change, such as acclimation or acclimatization, as well as learning. Nicotra *et al.* (2010) argue that, in the context of rapid climate change, phenotypic plasticity can be a crucial determinant of plant responses, both short- and long-term (Jump, 2009). There is general acceptance that high levels of genetic variation within natural populations improve the potential to withstand and adapt to novel biotic and abiotic environmental changes including the tolerance of climatic change.

Different concepts and definitions of stability have been described over the years (Lin *et al*., 1986; Becker and Léon, 1988). Producers are more interested in cultivars that are specifically adapted to their conditions and needs, with a high degree of stability over time but still with superior yield and quality. Stable and sustainable yields under varying environmental conditions have thus been gaining importance over increased yields

(Ceccarelli, 1989). Stability can be either static or dynamic (Becker and Léon 1988). Static stability implies that the performance of a genotype does not change under different conditions (specifically relevant for disease resistance). Dynamic stability entails that the genotype is affected by the environment but its relative performance is consistent across environments.

2.1.5 Analysis of G by E

For data sets with more than two genotypes and more than two environments, the GEI is commonly calculated by analysis of variance (ANOVA), leading to an estimated variance component for GEI (Annicchiarico, 2002).

Many statistical methods have been proposed by various researchers for the analysis of GEI (DeLacy *et al*., 1996). These include regression coefficient (Finlay and Wilkinson, 1963), sum of squared deviations from regression (Eberhart and Russell, 1966.), stability variance (Shukla, 1972), coefficient of determination (Pinthus, 1973), coefficient of variability (Francis and Kannenberg, 1978) and additive main effects and multiplicative interaction (AMMI) (Gauch and Zobel, 1988; 1996; Annicchiarico, 1997). The latest method that was developed is the GGE-biplot for graphical display of a GEI pattern (Yan, 1999; Yan *et al*., 2000).

The methods employed for the analysis of GEI can be classified into two major groups depending on the nature of the data available and the objectives of the analysis. The classical analysis of GEI involves exploiting yield-based data and evaluating genotypic performance across trials. Alternatively, it is often desirable to describe the reaction of genotypes to environments relative to the biophysical variables that directly affect crop yield for example to interpret GEI. Voltas *et al*. (2005) refer to these approaches as empirical or analytical strategies of G by E analysis.

2.2 SUMMARY

In citrus breeding, the existence of significant GEI of the crossover type can be a serious constraint to the improvement of citrus scions and rootstocks cultivars. Both of these genetically different plant parts are susceptible to environmental influences and to complicate matters further, there is an interaction between the scion and the rootstock deeming the one an environment of the other. This has a profound impact on both the breeding of new cultivars and the execution of cultivar trials for recommendations to producers regarding adaptability and stability. Although many papers deal with the evaluation of newly bred citrus scions and rootstock, an extensive search only yielded a few publications on citrus and GEI. The impact of climate change on fruit producing areas in South Africa is fast becoming a reality. Producers farming with fruit trees are particularly vulnerable as they invest substantial amounts of money into a fruit orchard from which they only start to reap the benefits after approximately eight years (break-even point). Citrus breeding and evaluation programmes should therefore rely on measuring GEI to select cultivars that will be able to adapt to climate change but still have a stable income.

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Chapter 3

CULTIVAR EVALUATION RELYING ON GENOTYPE MAIN EFFECT USING PREVIOUS PHASE II SCION CITRUS TRIALS AT THE ARC-ITSC

Abstract

The aim of this chapter was to study the intricacies of a scion-rootstock evaluation trial with regard to identifying a superior scion per locality over a number of years and the best performing scion and rootstock per locality using the typical univariate analysis applied by fruit tree researchers. In the South African breeding programmes, selections are made upon a primary screening (Phase I) of tree characteristics, external and internal fruit characteristics as well as fruit quality in one locality. This is followed by Phase II trials, which aim at testing superior genotypes selected from the ARC-ITSC breeding programmes, at different localities to acquire adequate information on their performance, in comparison with standard cultivars to warrant commercialisation and to facilitate cultivar recommendations. As the influence climate on yield and fruit quality in citrus is well documented, six independent trials were planned and implemented since 1988 at five different climatic zones in South Africa during Phase II. The six trials were based on the scion species used namely C. sinensis (Navels), C. sinensis (midseason), C. sinensis (Valencia), C. limon, C. paradisi and C. reticulate. A combined analysis of variance was performed annually per locality, Characteristics measured in the trials were tree size and volume, fruit production (yield), external fruit quality and internal fruit characteristics. The C. sinensis (Valencia) trial at Malelane was chosen to study the univariate approach for annual data analysis per locality and data analysis with regard to year effect on production and quality. The C. paradisi (grapefruit) trials at Malalane, Messina and Friedenheim were chosen to study the univariate approach for the comparison of genotypes per group over three localities for quality. The two trials resulted in 60 data tables representing data of 10 years. For the purpose of this chapter, nine of the 60 data tables were chosen to evaluate the univariate approach for data analysis of the Phase II stage of a breeding programme.

Regarding the Valencia group at Malalane all of the new cultivars were better than the standard Valencia cultivar (1043) and could be recommended and the best rootstock for yield was 575. The same applied to quality aspects where the standard Valencia cultivar (1043) constantly displayed the lowest quality. It was also concluded that the best rootstocks with regard to yield and quality were 575 and 608 respectively. However, GEI was evident and complex as illustrated by rank changes from one year to another as well as amongst three localities compared in the grapefruit trial and the high significance of GEI in the ANOVA over five years where E was years. GEI was evident for both scions and rootstocks. It was evident, in conclusion that the most commonly used univariate statistical tests such as ANOVA and t-tests for analysing GE interaction are not sufficient

to unravel the genotype x genotype interaction and its subsequent interactions with the environment. It was further concluded that in order to identify mega-environments within multi-environmental trials with a two-fold objective of identfying genotypes with both high performance and stability as well as test environments that are both representative and discriminative, multivariate models should be investigated with the available data for future application in a fruit-breeding programme. While an ordinary ANOVA sufficiently identify and test sources of variability, it does not express or describe patterns of the underlying interaction. Based on its additive nature an ordinary ANOVA description of main effects is possible but interactions are represented by the residual from the additive model, which is nonadditive and requires other techniques to identify the interaction relationships (Shaffi and Price, 1998).

3.1 INTRODUCTION

In South Africa citrus represents one of most important agro-commodities by value and by volume. Ever since the inception of the Citrus and Subtropical Research Institute now the Agricultural Research Council's Institute for Tropical and Subtropical Crops (ARC-ITSC) in 1926, it has been the mission to optimise the long-term global competitiveness of the Southern African citrus industry (Joubert, 1995). Breeding and cultivar evaluation has therefore been established as one of the strategic objectives of the ARC-ITSC to meet the dynamic cultivar requirements of the industry (Breedt *et al*., 1996). The ultimate goal of a plant breeder is to develop new genotypes that are superior, in one way or another, to the commercial cultivars available and that can be released as new cultivars to producers for commercial production. This objective will never change - the traits might. To attain this objective the genetic potential of breeding material needs to be assessed. However, it is impossible to assess genetic potential in isolation without taking the environment, in which the genetic material finds itself, into account.

To survive the highly competitive export domain, it is essential for citrus producers to expand on their cultivar basket (Ndou, 2012). However, this entails a high financial input from the producer and regarding citrus (as in any tree breeding), the yield and quality stability across years and environments of a newly bred cultivar is a major concern to many producers. Citrus is a long-term crop, usually not bearing fruit before two years after planting and with a financial break-even point of ± 8 years. Planting the wrong cabbage or maize cultivar may result in reduced profit, but planting the wrong citrus cultivar has serious long-term repercussions (Chadwick, 2010). All citrus growers are cognizant of the time it takes for an orchard to break even, and if the choice of cultivar is wrong, the financial consequences are dire.

Sinclair and Bartholomew (1944) discussed the influence of rootstocks as well as climate on fruit quality and advocated the use of different rootstocks in evaluation trials together with planting in different climate regions. When data are compared over localities/years the trials are deemed multi-environment trials (MET). These environments can be either artificial such as various levels of fertiliser or it can be natural such as different production seasons or climate zones.

Although data may be collected for many traits, analysis may be limited to a single trait (usually yield) and information on other traits is often left unexplored (Yan and Tinker, 2006). Furthermore, analysis of G x E data is often limited to genotype evaluation based on genotype main effect (G) while GEI is treated as noise or a confounding factor (Yan and Tinker, 2006).

Purely environmental effects, reflecting the different ecological potential of sites and management conditions, are not of direct concern for the breeding or recommendation of new cultivars. Genotypic main effects (i.e. differences in mean yield between genotypes) provide the only relevant information when GEI effects are absent or ignored. Extensive investigations by amongst others DeLacy *et al.* (1990) proved that differences between genotypes might vary widely among environments in the presence of GEI.

The aim of this chapter was to study the intricacies of a scion evaluation trial identifying improved genotypes, on standard industry rootstocks, over a number of years for a specific locality using the typical univariate analysis applied by fruit tree researchers.

3.2 MATERIALS AND METHODS

3.2.1 General background information

During the breeding phase (Figure 1.1), selections are made on a primary screening (Phase I) of tree characteristics, external and internal fruit characteristics as well as fruit quality. During Phase I screening, yield is excluded due to the impact of juvenility (Stover *et al*., 2011) and the vast number of single unique Phase I seedlings in the breeding programme at any given time (currently 17000 unique single seedlings). Individuals selected from Phase I are budded onto one or several commercial rootstocks and planted in replicated field trials (Phase II) in multiple localities representing the different climatic zones in South Africa. Phase II plantings can also include promising material from other breeding programmes in the world. The objectives of the Phase II stage of the protocol are to identify truly superior individual selections and to acquire adequate information on their performance, in comparison with standard commercial cultivars, to warrant further development leading to possible release of new cultivars (Breedt *et al.*, 1996, Gmitter *et al*., 2007). In South Africa the Phase II scion and rootstock trials were planted at five different localities as classified by Barry (1996) namely hot humid (Malalane), intermediate (Friedenheim), hot dry (Messina) and two cold regions (Addo and Citrusdal).

Due to the diversity of the *Citrus* genus as explained in Chapter 2, scion trials at each of the five localities comprised of six different trials (orchards). The scion species encompassed *C. sinensis* (three types: Navels, Midseason and Valencia), *C. limon, C. paradisi* and *C. reticulate* and at the cold production area, *C. unshi* was included (Table 3.1).

Table 3.1 A summary of controls and number of citrus genotypes per citrus type included in various Phase II trials at the ARC-ITSC between 1988 and 2002

Citrus scion type	Number of genotypes included	Control cultivar
C. sinensis - Navels	44	'Palmer' (cv. no.1072)
C. sinensis - midseason	39	'Tomango' (cv. no.1047)
C. sinensis - Valencia	45	'Delta' (cv. no.1043)
C. paradisi - grapefruit	17	'Marsh' (cv. no. 1057)
C. limon - lemons	31	'Eureka'(cv. no. 1073)
C. mandarin - mandarins	45	'Ellendale' (cv. no. Beauty 228) 'Clementine' (cv. no. 1048)

As initially intended, data for each trial at each locality was individually analysed and reported annually. Two data tables (yield and quality) were generated per *Citrus spp.* per year. Only a few data tables from this vast number were extracted to study the pros and cons of the univariate approach..

3.2.2 Localities

For the purpose of this study, data of three of the localities were included namely hot humid (Malalane), intermediate (Friedenheim) and hot dry (Messina) as classified by Barry (1996). The coordinates in decimal degrees are -25.463667, 31.587667 for Malalane, -

22.201194, 29.884556 for Messina and -25.443, 30.9889972 for Friedenheim. The soil at Malalane is a very shallow Mispah type while at Messina it varies from a light Hutton (5-15% clay) to a light Oakleaf is (5-10% clay). Soil types at the Friedenheim trial site consists of a Hutton-Mesinga series (15-35 %).

3.2.3 Materials

3.2.3.1 Scion material

To evaluate the univariate approach for annual data analysis per locality and data analysis with regard to year effect on production and quality, the Valencia group was chosen with five selections namely Midknight (1044), Valencia Late selection (1052), Olinda (1056), Du Roi (1060), Valencia Late selection (1063) and the control cultivar Delta (1043). Valencia was chosen as it was the most widely planted citrus group in South Africa.

In order to evaluate the univariate approach for the comparison of genotypes per group over three localities for quality, grapefruit was used as the study material and included six selections namely Ruby Blush (231), Marsh selection (1053), Redblush (1058), Nartia (1059), Marsh selection (1176), Star Ruby (1179) and Marsh (1057) as control.

3.2.3.2 Rootstock used

Albeit a scion trial, the scions were grafted onto four different commercially available rootstocks namely Van Stadens Rough lemon (*C. jambhiri,* Tenaka)*,* Volckamer lemon (*Citrus volkameriana* V. Ten. & Pasq.), Empress Rosehaugh (*C. reticulate* Swingle*)* and Carrizo citrange (*Poncirus trifoliate* x *C. sinensis)*. For the benefit of graphs and tables, the respective cultivar numbers in the ARC-ITSC gene bank for these rootstocks namely 42, 575, 306 and 608 were used. Malalane was the only locality where trees on all four rootstocks survived for the total duration of the trial. At Messina, for instance, few trees survived on 'Rosehaugh Empress', data from Malalane was thus used for further statistical analysis.

3.2.4 Methods

3.2.4.1 Virus cleansing, cross protection and nursery practices

In accordance to the statutory South African Citrus Improvement Scheme (SA-CIS) scion cultivars selected to be planted in Phase II trials were proven virus free after shoot-tip grafting and pre-immunised before it was grafted onto the relevant rootstocks.

Rootstock cultivars are propagated as seedlings due to their poly-embryonic nature. Seedling rootstocks were budded at a standard height of 300 mm with buds taken from the pre-immunised plants. Trees were kept in vector-free tunnels in the area where the field trials were to be planted until buds have grown out and the plants conformed to the standard requirement for citrus nursery trees.

3.2.4.2 Production practises

Production practises were implemented specific with regard to the type of citrus (in this case Valencia and grapefruit) and kept optimal as far as possible. Fertilisers were applied according to soil and leaf sample analysis. Pest and disease control followed the standard programme as annually prescribed by the citrus industry. Irrigation was by means of micro-sprinklers and scheduled according to the prescribed crop factors.

3.2.4.3 Trial layout

Due to production requirements, Valencia and grapefruit were planted in separate orchards. The trial layout per orchard was a randomised block design with 16 trees per scion genotype consisting of four trees budded for each of the different rootstocks. Trees were planted at an orchard spacing of 7 m x 3 m. The Valencia trial consisted of 24 treatment combinations (six scions and four rootstocks), replicated four times with each replication consisting out of one tree thus 96 trees. For the Grapefruit trial, 28 treatment combinations (seven scions and four rootstocks) were used and were replicated four times with each replication consisting out of one tree thus 112 trees. The same trial layout was used at three different locations thus 336 trees in total.

3.2.4.4 Evaluation of traits

A budded citrus tree generally starts bearing within two years after planting. Evaluation is done annually as soon as the fruit reaches harvest maturity. The tree and fruit evaluations are done according to standard norms (Hardy and Sanderson, 2010; Van Rensburg, 1985; Wardowski *et al*., 1995). The characteristics that were measured in the trials were fruit production (yield), external fruit quality (size, colour, texture, fruit size distribution) and internal fruit characteristics (fruit weight, juice weight, peel thickness, total soluble solids (TSS), titratable acid (TA) and seed quantity). The measured traits were used to determine fruit quality (fruit mass, juice %, TSS:TA ratio and peel thickness).

3.2.5 Data analysis

3.2.5.1 Annual data analysis per locality using Valencia data at Malalane as example

A combined ANOVA was performed annually per locality, using PROC GLM of the SAS/STATsoftware, Version 6.0 of the SAS System for [Unix]. SAS Institute Inc. (1990). The Shapiro-Wilk test was performed to test for normality of the residuals (Shapiro and Wilk, 1965). Treatment means (e.g. scion, rootstock, scion x rootstock) were compared using Fisher's t test with LSD (Steel and Torrie, 1980).

Yield in most types of citrus increases annually for eight years where after it stays constant (Hearn, 1981; Soost and Cameron, 1981). Data was thus taken for ten consecutive years commencing from the first year after plant. Two data tables (yield and quality) were generated per trial per year for ten years, culminating in 20 tables for instance for Valencia. Due to low yields, quality data on some of the rootstocks could only be recorded from year four. Thus starting from year four for three consecutive years, six of the 20 tables (three for yield and three for quality) were chosen to study the univariate method.

3.2.5.2 Comparing data tables per group amongst three localities for a single year using grapefruits as an example

Commencing from the second year after planting, data were taken, analysed and reported annually for ten consecutive years. This approach only accounted for the genotypic main effects and due to the inclusion of more than one rootstock, gave an indication of the scion rootstock interaction. Data tables from three localities were compared side by side. The three localities for this purpose are Malalane, Friedenheim and Messina.

Data tables were compiled using a combined ANOVA was performed using PROC GLM of the SAS/STAT software, Version 8 of the SAS System (SAS Institute Inc. 1999). The Shapiro-Wilk test was performed to test for normality of the residuals (Shapiro and Wilk, 1965). Treatment means were compared using Fisher's t test (P=0.05) with Least Significant Difference (LSD) was calculated at 5% significance level to compare treatment means (Steel and Torrie, 1980).

3.2.5.3 Comparing data per group in one locality over five years with Valencia as an example

The majority of citrus being produced in South Africa is Valencia types, therefore to illustrate the year effect with regard to yield, the data for Valencia from Malalane for five consecutive years on four rootstocks was used for the analysis.

The experimental design was a randomised block design and a split-plot treatment design. According to Little and Hills (1972) a split-plot principle can be applied to experiments where successive observations are made on the whole units (rootstocks) over time (years). Levene's test for homogeneity of variance was performed to test the seasonal variability in observations for comparable magnitude (Levene, 1960).

Combined ANOVA was performed using PROC GLM of the SAS/STATsoftware, Version 12.1 of the SAS System for [Unix]. (SAS Institute Inc. 2012). The Shapiro-Wilk test was performed to test for normality of the residuals (Shapiro and Wilk, 1965). Treatment means were compared using Fisher's t test (P=0.05) with Least Significant Difference (LSD) was calculated at 5% significance level to compare treatment means (Steel and Torrie, 1980).

3.3 RESULTS

3.3.1 Annual data analysis of yield per single locality using data of the Valencia trial at Malalane

The three selected annual data tables with regard to the yield performance (total production=kg tree^{-1}) and fruit size of Valencia as a percentage of fruit per count (count = number of fruit per 15 kg export container) in one locality for Valencias on four rootstocks are depicted in Tables 3.2 to 3.4.

Scions: In the first season (Table 3.2), the control cultivar 1043 yielded the lowest at 15.2 kg tree^{-1}. The yield was significantly lower than the yield of selection 1056 (33.8 kg tree^{-1}) but not significantly lower than the other selections. Selection 1044 and 1056 had the largest fruit size with respectively 82.1 and 80.9% of the fruit in a 15 kg carton being larger than 90 mm (count >40).

There were two significantly different groups regarding yield (total production), in the second season (Table 3.3) with 1056, 1063, 1052, 1060 and 1055 being significantly better than 1043 and 1044. Selection 1056 remained the best performer (80.7 kg $tree^{-1}$) but did not differ significantly from the other selections in the top five performers. Fruit size of selection 1044 were significantly larger than that of the other selections with 48.8% of the fruit in a 15 kg carton being larger than 90 mm (count >40).

In general, the production in the third season with a trial mean of 48.1 kg $tree^{-1}$ (Table 3.4) was lower than in the second season, which had a trial mean of 65.2 kg $tree^{-1}$ (Table 3.3) for total production. Compared to the second season, only 1043 had a higher yield in the third season but were still together with 1044 ranking the lowest with regard to total production. Selection 1044 yielded significantly lower than all the other Valencia selections. Selection 1056 yielded the highest (62.7 kg $tree^{-1}$) but did not differ significantly from selection 1063, 1060, and 1052.

Rootstocks : In the first season the trial mean of 62.1% for count >40 indicated that small fruit was not a problem (Table 3.2). The rootstock that performed the best with regard to yield in the first season was 575 (37.4 kg $tree^{-1}$). Fruits of trees on rootstock 42 were significantly larger than fruit of trees on the other three rootstocks. In the second season (Table 3.3), trees on rootstock 575 had significantly higher yields than trees on the other rootstocks. In the third season (Table 3.4), rootstock 575 again statistically outperformed trees on the other rootstocks, while trees on rootstock 306 were significantly lower yielding than trees on the other three rootstocks.

Interaction between scions and rootstocks: The interaction between Valencia selections and rootstocks for yield was only significant in the six-year-old trees. For fruit-size distribution, the interaction was highly significant in the second for counts 88, 72 and 40, while in the third season on six year old trees the rootstock-scion interaction for fruit-size distribution were only significant for counts 56 and 48. Scion x rootstock interaction with regard to total production was non-significant for the first and second season (Table 3.2 and 3.3) but highly significant on the six-year-old trees in the third season (Table 3.4).

Table 3.2 Production and fruit size distribution of Valencia genotypes in combination with four rootstocks harvested at Malalane on four-year-old trees

no	Genotype	Fruit distribution given as the percentage of fruit per count#								Total production	
	Scions	Count	≤72 (%)	Count 56 (%)		Count 48 (%)		Count >40 (%)		(kg.tree^{-1})	
1043	Delta(Control)	**1063**	1.3 a	**1060**	23.3 a	**1060**	32.0 a	**1044**	82.1 a	**1056**	33.8 a
1044	Midknight	**1043**	1.2 a	**1063**	16.9 ab	**1063**	27.5 ab	**1056**	80.9 a	**1060**	29.3 ab
1052	Valencia Late	**1052**	1.0 a	**1052**	16.2 ab	**1055**	23.6 abc	**1043**	67.9 ab	**1052**	24.6 ab
1055	Olinda	**1055**	0.9 a	**1055**	15.8 ab	**1052**	20.1 bcd	**1052**	62.8 bc	**1063**	20.2 ab
1056	McClean	**1060**	0.7 a	**1043**	12.9 ab	**1043**	18.0 bcd	**1055**	59.8 bc	**1055**	19.7 ab
1060	Du Roi	**1044**	0.4 a	**1056**	6.2 b	**1056**	12.8 cd	**1063**	54.3 bc	**1044**	18.3 ab
1063	Valencia Late	**1056**	0.1 a	**1044**	4.9 b	**1044**	11.6 d	**1060**	44.1 c	**1043**	15.2 b
	Rootstocks	Count	≤72 (%)	Count 56 (%)		Count 48 (%)		Count >40 (%)		(kg.tree-1)	
575	Volckameriana	**575**	1.7 a	**306**	23.0 a	**608**	27.7 a	**42**	78.4 a	**575**	37.4 a
42	Van Staden	**608**	0.6 a	**608**	16.6 a	**306**	26.1 a	**575**	60.8 b	**608**	18.6 b
608	Carrizo Citrange	**42**	0.4 a	**575**	15.3 a	**575**	22.2 a	**608**	55.2 b	**42**	16.7 b
306	Rosehaugh Empress	**306**	0.2 a	**42**	6.6 b	**42**	14.6 b	**306**	50.7 b	**306**	9.2 b
Trial mean (x)		0.9		14.8		22.2		62.1		23.2	
Coefficient of variance %		210.5		67.7		40		26.9		50.1	
P Scion		0.5771 NS		0.0122*		0.0001***		0.0001***		0.0008***	
P Rootstock		0.0245*		0.00281***		0.0031***		0.0009***		0.0001***	
P Scion*Rootstock		0.4672 NS		0.6590 NS		0.0698 NS		0.1716 NS		0.6195 NS	

Values per trait followed by the same alphabetically letter did not differ significantly at P≤0.05
Count = Number of fruit per 15 kg container
* P≤0.05, *** P≤0.01 and NS=Non significant

Table 3.3 Production and fruit size distribution of Valencia cultivars in combination with four rootstocks harvested at Malalane on five-year-old trees

no	Genotype	Fruit distribution given as the percentage of fruit per count[#]														Total production	
Scions		Count ≤112 (%)		Count 88 (%)		Count ≤72 (%)		Count 56 (%)		Count 48 (%)		Count 40 (%)		Count >40 (%)		(kg.tree-1)	
1043	Delta(Control)	**1055**	3.9 a	**1055**	8.9 a	**1055**	40.8 a	**1063**	21.7 a	**1043**	40.4 a	**1044**	32.4 a	**1044**	48.8 a	**1056**	80.7 a
1044	Midknight	**1063**	3.1 a	**1063**	5.8 ab	**1044**	32.8 a	**1052**	21.6 a	**1052**	23.3 b	**1043**	20.7 b	**1056**	7.1 b	**1063**	69.8 a
1052	Valencia Late	**1060**	1.8 a	**1060**	5.3 ab	**1052**	31.6 a	**1056**	20.6 a	**1056**	23.1 b	**1060**	17.5 bc	**1060**	5.7 b	**1052**	69.5 a
1055	Olinda	**1052**	1.6 a	**1052**	3.9 ab	**1056**	30.7 a	**1060**	18.4 a	**1063**	22.2 b	**1056**	14.8 bc	**1052**	4.5 b	**1060**	66.3 a
1056	McClean	**1043**	0.7 a	**1056**	3.0 ab	**1060**	29.5 a	**1055**	16.6 a	**1060**	21.7 b	**1052**	13.6 bc	**1055**	3.7 b	**1055**	64.6 a
1060	Du Roi	**1056**	0.6 a	**1043**	1.9 b	**1043**	16.9 b	**1043**	15.9 a	**1055**	17.4 b	**1063**	10.5 bc	**1043**	3.5 b	**1043**	29.5 b
1063	Valencia Late	**1044**	0.0 a	**1044**	0.2 b	**1044**	1.0 c	**1044**	3.6 b	**1044**	14.1 b	**1055**	8.6 c	**1063**	2.2 b	**1044**	23.4 b
Rootstocks		Count ≤112 (%)		Count 88 (%)		Count ≤72 (%)		Count 56 (%)		Count 48 (%)		Count 40 (%)		Count > 40 (%)		(kg.tree-1)	
575	Volckameriana	**42**	4.0 a	**42**	7.6 a	**306**	40.1 a	**608**	21.0 a	**575**	26.0 a	**575**	19.6 a	**608**	10.2 a	**575**	90.0 a
42	Van Staden	**306**	2.9 a	**306**	7.4 a	**42**	34.5 a	**306**	20.1 a	**608**	23.8 a	**608**	15.5 ab	**575**	6.9 a	**42**	69.2 b
608	Carrizo Citrange	**575**	0.8 b	**608**	2.8 b	**608**	26.0 b	**575**	19.2 ab	**306**	22.5 ab	**42**	13.6 b	**42**	6.7 a	**306**	48.8 c
306	Rosehaugh Empress	**608**	0.7 b	**575**	2.5 b	**575**	25.0 b	**42**	15.7 b	**42**	17.9 b	**306**	6.1 c	**306**	0.8 b	**608**	44.1 c
Trial mean (x)		2.1		4.9		30.9		18.9		22.7		14.2		6.3		65.2	
Coefficient of variance %		151		95.2		40.8		32.7		36.8		59.2		110		26.2	
P Scion		0.1221 NS		0.0077***		0.0001***		0.0001***		0.0001***		0.0001***		0.0001***		0.0004***	
P Rootstock		0.0047***		0.0007***		0.0001***		0.0001***		0.0194*		0.0001***		0.0001***		0.0001***	
P Scion*Rootstock		0.1187 NS		0.0018***		0.0026***		0.3566 NS		0.0170*		0.0048***		0.2024 NS		0.1067 NS	

Values per trait followed by the same alphabetically letter did not differ significantly at P≤0.05.
Count = Number of fruit per 15 kg container
* P≤0.05, *** P≤0.01 and NS=Non significant

Table 3.4 Production and fruit size distribution of Valencia cultivars in combination with four rootstocks harvested at Malalane on six-year-old trees

no	Genotype	Fruit distribution given as the percentage of fruit per count														Total production	
	Scions	Count	≤112 (%)	Count	88 (%)	Count	≤72 (%)	Count	56 (%)	Count	48 (%)	Count	40 (%)	Count	>40 (%)		(kg.tree-1)
1043	Delta(Control)	**1052**	0.4 a	**1052**	3.4 a	**1060**	19.3 a	**1052**	22.1 a	**1055**	48.3 a	**1044**	21.2 a	**1044**	49.3 a	**1056**	62.7 a
1044	Midknight	**1060**	0.3 a	**1056**	2.0 ab	**1063**	16.7 ab	**1060**	22.0 a	**1043**	46.2 ab	**1052**	20.9 a	**1055**	20.6 b	**1063**	60.2 ab
1052	Valencia Late	**1056**	0.3 a	**1063**	1.0 bc	**1056**	14.4 abc	**1063**	20.6 a	**1060**	43.5 ab	**1063**	17.6 ab	**1056**	15.3 bc	**1060**	58.1 abc
1055	Olinda	**1063**	0.2 a	**1060**	0.9 bc	**1052**	11.7 abc	**1043**	17.9 ab	**1056**	41.6 abc	**1043**	10.9 bc	**1043**	14.7 bc	**1052**	50.6 abc
1056	McClean	**1055**	0.1 a	**1043**	0.6 bc	**1043**	9.6 bc	**1056**	17.5 ab	**1063**	39.5 bc	**1055**	9.5 c	**1060**	9.5 cd	**1055**	49.4 bc
1060	Du Roi	**1043**	0.1 a	**1055**	0.4 bc	**1055**	7.4 cd	**1055**	13.7 b	**1052**	34.6 c	**1056**	8.9 c	**1052**	7.0 cd	**1043**	47.5 c
1063	Valencia Late	**1044**	0.0 a	**1044**	0.0 c	**1044**	0.9 d	**1044**	1.7 c	**1044**	27.0 c	**1060**	4.5 c	**1063**	4.4 d	**1044**	12.1 d
	Rootstocks	Count	≤112 (%)	Count	88 (%)	Count	≤72 (%)	Count	56 (%)	Count	48 (%)	Count	40 (%)	Count	> 40 (%)		(kg.tree-1)
575	Volckameriana	**42**	0.6 a	**42**	2.7 a	**42**	15.9 a	**42**	20.5 a	**306**	45.6 a	**575**	18.9 a	**575**	19.7 a	**575**	68.5 a
42	Van Staden	**306**	0.1 b	**608**	0.9 b	**306**	13.8 a	**306**	18.0 a	**575**	42.4 a	**608**	11.7 b	**608**	17.3 ab	**42**	57.2 b
608	Carrizo Citrange	**575**	0.0 b	**306**	0.8 b	**608**	11.1 ab	**608**	17.4 a	**608**	41.6 a	**42**	10.9 b	**42**	16.3 ab	**608**	38.9 c
306	Rosehaugh Empress	**608**	0.0 b	**575**	0.3 b	**575**	6.4 b	**575**	12.2 b	**42**	33.1 b	**306**	10.6 b	**306**	11.1 b	**306**	25.2 d
Trial mean (x)		0.2		1.2		11.6		16.8		40.5		13.3		16.4		48.1	
Coefficient of variance %		244		205		87.4		45.1		22.9		67.3		65		33.9	
P Scion		0.3533NS		0.0130*		0.0006***		0.0001***		0.0001***		0.0001***		0.0001***		0.0001***	
P Rootstock		0.0003***		0.0103*		0.0160*		0.0032***		0.0007***		0.0050***		0.4468 NS		0.0001***	
P Scion*Rootstock		0.6226 NS		0.1448 NS		0.1555 NS		0.0205*		0.0163*		0.1451 NS		0.0605 NS		0.0007***	

Values per trait followed by the same alphabetically letter did not differ significantly at P≤0.05.
Count = Number of fruit per 15 kg container
* P≤0.05, *** P≤0.01 and NS=Non significant

3.3.2 Annual data analysis of quality per single locality using Valencia data at Malalane

Quality data of the same genotype combinations as in Table 3.2 to Table 3.4 are given in Table 3.5 to Table 3.7.

Scions: Significant differences were recorded on the four-year-old trees (Table 3.5) for all the quality aspects except rag strength for both the scion and the rootstocks. With regard to peel thickness in Valencia, the control cultivar (1043) was the least sought after cultivar with significantly thicker peels and the lowest values for TSS and acid. With regard to juice percentage, selections 1043 and 1044 were significantly lower than the other selections but did not differ significantly from each other. The control cultivar (1043) together with selections 1044 and 1056 had the thickest peels and ranked the lowest in TSS and acid. Cultivar 1043 and selection 1044 did not attain the minimum export requirement for juice content of 50% and were significantly lower than the other cultivars. None of the scions achieved the minimum export standard for TSS of 9.0%.

Although all the scions in season two and three (Table 3.6 and 3.7) met the export standards for juice content and TSS, selection 1060 and control cultivar 1043 had significantly lower TSS and acid percentages than the other five selections evaluated. In season three all the selections had a peel thickness significantly thinner than that of the control cultivar (1043).

Rootstocks: Fruit characteristics, in seasons one and three, on rootstock 608 and 306 were significantly better with regard to juice percentage and TSS than on rootstocks 42 and 575.

Interaction between scions and rootstocks: Interaction between rootstocks and scions of the four-year-old trees was not significant for juice content, TSS percentage and rag strength. In the second season, the interaction between rootstocks and scions were significant for all quality aspects measured. Contrary to this, the interaction between rootstocks and scions were not significant for any of the quality aspects measured in the third season (Table 3.7). In both season two and three, rootstocks 608 and 306 ended predominantly in the top positions.

Table 3.5 Quality of Valencia cultivars in combination with four rootstocks harvested at Malalane from four-year-old trees

no	Genotype	Fruit quality traits											
	Scions	**Juice %**		**Peel (mm)**		**TSS %**		**Acid %**		**Ratio**		**Rag strenght %**	
1043	Delta(Control)	**1063**	54.4 a	**1055**	4.7 c	**1055**	7.6 a	**1055**	0.93 a	**1043**	10.8 a	**1052**	46.2 a
1044	Midknight	**1055**	54.1 a	**1060**	4.7 c	**1063**	7.5 a	**1063**	0.86 b	**1044**	10.1 ab	**1044**	47.3 a
1052	Valencia Late	**1060**	53.2 a	**1063**	5.0 bc	**1052**	7.4 ab	**1052**	0.81 bc	**1056**	9.6 bc	**1060**	47.5 a
1055	Olinda	**1056**	52.8 a	**1052**	5.1 bc	**1060**	7.2 ab	**1060**	0.78 cd	**1060**	9.5 bc	**1043**	47.7 a
1056	McClean	**1052**	52.4 a	**1044**	5.2 abc	**1044**	6.9 abc	**1044**	0.73 de	**1052**	9.4 bc	**1056**	48.2 a
1060	Du Roi	**1043**	49.8 b	**1056**	5.4 ab	**1056**	6.7 bc	**1056**	0.71 e	**1063**	8.9 c	**1055**	48.3 a
1063	Valencia Late	**1044**	49.2 b	**1043**	5.7 a	**1043**	6.5 c	**1043**	0.63 f	**1055**	8.2 d	**1063**	49.3 a
	Rootstocks	**Juice %**		**Peel (mm)**		**TSS %**		**Acid %**		**Ratio**		**Rag strenght %**	
575	Volckameriana	**306**	55.2 a	**608**	4.8 b	**306**	8.0 a	**306**	0.91 a	**608**	10.4 a	**575**	46.5 a
42	Van Staden	**608**	54.7 a	**306**	4.9 b	**608**	7.8 a	**608**	0.78 b	**575**	9.2 b	**306**	47.5 a
608	Carrizo Citrange	**42**	51.5 b	**575**	5.1 ab	**575**	6.8 b	**575**	0.77 b	**42**	9.0 b	**42**	48.8 a
306	Rosehaugh Empress	**575**	51.3 b	**42**	5.4 a	**42**	6.5 b	**42**	0.75 b	**306**	9.0 b	**608**	49.6 a
Trial mean (x)		52.7		5.1		7.2		0.79		9.4		47.9	
Coefficient of variance %		3.3		9.8		7.5		8.1		7.4		11.2	
P Scion		0.0001***		0.0001***		0.0001***		0.0001***		0.0001***		0.9513 NS	
P Rootstock		0.0001***		0.0001***		0.0001***		0.0001***		0.0001***		0.3115 NS	
P Scion x Rootstock		0.0663NS		0.0470*		0.4931 NS		0.0116***		0.0219***		0.8289 NS	

Values per trait followed by the same alphabetically letter did not differ significantly at P≤0.05.
* P≤0.05, *** P≤0.01 and NS=Non significant

Table 3.6 Quality of Valencia cultivars in combination with four rootstocks harvested at Malalane from five-year-old trees

no	Genotype	Fruit quality traits										
	Scions		**Juice %**		**Peel (mm)**		**TSS %**		**Acid %**		**Ratio**	**Rag strenght %**
1043	Delta(Control)	**1063**	58.0 a	**1044**	4.9 b	**1044**	11.0 a	**1055**	1.5 a	**1044**	11.2 a	**No data**
1044	Midknight	**1043**	55.6 ab	**1060**	5.0 b	**1055**	10.9 a	**1063**	1.3 b	**1043**	10.8 a	
1052	Valencia Late	**1052**	54.6 ab	**1055**	5.1 ab	**1056**	10.5 ab	**1056**	1.2 c	**1052**	9.0 b	
1055	Olinda	**1055**	53.3 ab	**1063**	5.2 ab	**1063**	10.4 abc	**1052**	1.1 c	**1056**	8.7 bc	
1056	McClean	**1056**	52.4 b	**1056**	5.2 ab	**1052**	10.3 bc	**1060**	1.1 c	**1060**	8.6 bc	
1060	Du Roi	**1060**	51.4 b	**1052**	5.2 ab	**1043**	9.9 c	**1044**	1.0 d	**1063**	8.0 cd	
1063	Valencia Late	**1044**	50.8 b	**1043**	5.4 a	**1060**	9.8 c	**1043**	0.9 d	**1055**	7.4 d	
	Rootstocks		**Juice %**		**Peel (mm)**		**TSS %**		**Acid %**		**Ratio**	**Rag strenght %**
575	Volckameriana	**306**	59.3 a	**306**	4.9 b	**608**	11.1 a	**306**	1.3 a	**608**	9.6 a	
42	Van Staden	**608**	53.9 b	**608**	5.0 b	**306**	11.1 a	**42**	1.2 b	**306**	8.9 b	
608	Carrizo Citrange	**42**	52.9 b	**575**	5.3 a	**42**	10.2 b	**608**	1.2 b	**575**	8.7 b	
306	Rosehaugh Empress	**575**	50.8 b	**42**	5.4 a	**575**	9.4 c	**575**	1.1 c	**42**	8.7 b	
Trial mean (x)			54		5.2		10.4		1.19		9	
Coefficient of variance %			9.3		5.6		5.7		8.1		9.7	
P Scion			0.0106*		0.0012***		0.0001***		0.0001***		0.0001***	
P Rootstock			0.0001***		0.0001***		0.0001***		0.0001***		0.0679 NS	
P Scion x Rootstock			0.0498*		0.0498*		0.0341*		0.0002***		0.0019***	

Values per trait followed by the same alphabetically letter did not differ significantly at P≤0.05.
* P≤0.05, *** P≤0.01 and NS=Non significant

Table 3.7 Quality of Valencia cultivars in combination with four rootstocks harvested at Malalane from six-year-old trees

no	Genotype	Fruit quality traits											
	Scions	**Juice %**		**Peel (mm)**		**TSS %**		**Acid %**		**Ratio**		**Rag strenght %**	
1043	Delta(Control)	**1056**	56.4 a	**1052**	4.7 b	**1056**	10.9 a	**1055**	1.31 a	**1044**	12.2 a	**1044**	35.0 c
1044	Midknight	**1063**	56.0 a	**1044**	4.8 b	**1052**	10.8 ab	**1060**	1.23 ab	**1043**	11.9 a	**1043**	37.6 bc
1052	Valencia Late	**1055**	55.4 ab	**1055**	4.8 b	**1055**	10.8 ab	**1063**	1.21 b	**1052**	10.2 b	**1052**	38.4 bc
1055	Olinda	**1060**	55.4 ab	**1056**	4.9 b	**1044**	10.5 abc	**1056**	1.20 b	**1056**	9.3 bc	**1063**	40.6 ab
1056	McClean	**1043**	54.5 b	**1060**	4.9 b	**1063**	10.4 bcd	**1052**	1.08 c	**1063**	8.7 cd	**1055**	41.9 ab
1060	Du Roi	**1052**	54.4 b	**1063**	4.9 b	**1060**	10.0 cd	**1044**	0.87 d	**1055**	8.3 d	**1060**	42.3 ab
1063	Valencia Late	**1044**	51.7 c	**1043**	5.2 a	**1043**	9.9 d	**1043**	0.86 d	**1060**	8.2 d	**1056**	43.9 a
	Rootstocks	**Juice %**		**Peel (mm)**		**TSS %**		**Acid %**		**Ratio**		**Rag strenght %**	
575	Volckameriana	**306**	56.3 a	**608**	4.6 b	**306**	10.9 a	**42**	1.27 a	**608**	10.7 a	**608**	37.4 b
42	Van Staden	**608**	56.0 a	**306**	4.7 b	**608**	10.6 a	**306**	1.14 b	**575**	10.0 ab	**306**	38.5 b
608	Carrizo Citrange	**575**	54.0 b	**42**	5.0 a	**42**	10.6 a	**575**	1.05 c	**306**	9.8 b	**575**	38.6 b
306	Rosehaugh Empress	**42**	54.0 b	**575**	5.1 a	**575**	10.0 b	**608**	1.03 c	**42**	8.6 c	**42**	45.7 a
Trial mean (x)		4.9		55		10.5		1.12		9.7		40.2	
Coefficient of variance %		6.6		3		5		11.5		13		15.4	
P Scion		0.0235*		0.0001***		0.0002***		0.0001***		0.0001***		0.0087***	
P Rootstock		0.0001***		0.0001***		0.0001***		0.0001***		0.0001***		0.0001***	
P Scion x Rootstock		0.1715 NS		0.1141 NS		0.5923 NS		0.5411 NS		0.6306 NS		0.4660 NS	

Values per trait followed by the same alphabetically letter did not differ significantly at P≤0.05.
* P≤0.05, *** P≤0.01 and NS=Non significant

3.3.3 Comparison of data tables per group amongst three localities for a single year using grapefruits as an example

Data tables from three localities were compare side by side within and between localities. Table 3.8 represents data tables taken of four-year-old trees from three localities (Malalane, Friedenheim and Messina). In order to accommodate yield and mass in one table together with the quality features, fruit size distribution was omitted for the purpose of this comparison. The trees yielded a first crop in the third year after planting hence the data in Table 3.8 represents the second year of bearing. A noticeable difference in yield were observed amongst the three localities with Malalane having the highest values and Friedenheim having the lower values.

Average mean juice percentage at Friedenheim (Table 3.9) were lower than that of Malalane with all the cultivars reaching the minimum standard of 42% juice for the white and pink cultivars and selection 1179 realising the minimum juice percentage of 50% for red grapefruit cultivars (Table 3.8). Although selection 231 at Messina was able to exceed the minimum standard for juice percentage of 42%, it did not differ significantly from the other cultivars under investigation, which was way below the standard. Juice percentage (36.9%) for 1179 was also way below the standard of 50% at Messina. In comparison with all the grapefruit selections tested, 1179 had significantly softer rag at both localities.

With regard to rootstocks, 575 gave the highest yields in all the localities except at Friedenheim where 608 had a higher yield, which did not differ significantly from the second highest yield recorded on 575.

At Malalane and Friedenheim the interaction between scion and rootstock was not significant for most traits on four year old trees except for % TSS at Malalane and yield at Friedenheim (Table 3.9). The different scion genotypes did also not differ significantly from each other at Friedenheim and at Messina the yield, peel thickness and TSS of the scion genotypes differed significantly from each other. With regard to the interaction between scion and rootstock in the three localities, the only significant interaction was recorded in yield at Friedenheim. At Messina the rootstock and scion had no significant influence on the quality traits but did have an influence on yield. Table 3.9 represents the average means per trait over rootstocks and scions per locality.

Table 3.8 Yield and quality comparison of grapefruit cultivars in combination with four rootstocks harvested independently at Malalane, Friedenheim and Messina from four-year-old trees

No	Genotype	Yield		Juice		Peel		TSS		Acid		Ratio		Rag strength	
MALALANE (HOT HUMID)															
	Scion Key	No	(Kg/tree)	**No**	%	**No**	mm	**No**	%	**No**	%	**No**	%	**No**	%
231	Ruby Blush	**231**	36.2 a	**1179**	51.1 a	**1179**	7.8 b	**1058**	6.5 a	**1058**	1.13 a	**231**	6.0 a	**1057**	40.0 c
1053	Marsh	**1179**	36.0 a	**1058**	49.2 ab	**1057**	8.0 ab	**1179**	6.4 a	**1176**	1.11 a	**1069**	6.0 a	**1058**	41.6 bc
1057	Marsh (Control)	**1069**	34.9 a	**231**	48.8 ab	**1058**	8.1 ab	**1069**	6.4 a	**1057**	1.11 ab	**1179**	5.9 a	**1053**	45.1 abc
1058	Redblush	**1053**	32.2 ab	**1057**	48.5 ab	**231**	8.4 ab	**1057**	6.3 a	**1179**	1.08 ab	**1053**	5.8 a	**1176**	45.3 abc
1069	Nartia	**1176**	30.6 ab	**1176**	47.2 b	**1053**	8.4 ab	**231**	6.3 ab	**1053**	1.07 ab	**1058**	5.8 a	**1069**	45.4 abc
1176	Marsh	**1058**	23.5 b	**1053**	47.1 b	**1176**	8.8 a	**1053**	6.2 ab	**1069**	1.07 ab	**1057**	5.7 a	**231**	46.7 ab
1179	Star Ruby	**1057**	21.3 b	**1069**	46.9 b	**1069**	9.0 a	**1176**	5.9 b	**231**	1.04 b	**1176**	5.3 b	**1179**	48.1 a
	Rootstock keys														
575	Volckameriana	**575**	60.0 a	**306**	49.7 a	**306**	7.8 b	**306**	7.0 a	**306**	1.16 a	**608**	6.1 a	**306**	41.3 c
42	Van Staden	**608**	34.2 b	**575**	48.8 a	**608**	8.0 b	**608**	6.8 a	**608**	1.12 b	**306**	6.0 a	**608**	43.2 bc
608	Carrizo Citrange	**42**	15.0 c	**608**	48.7 a	**575**	8.3 b	**575**	5.8 b	**42**	1.06 c	**575**	5.7 b	**575**	46.0 ab
306	Rosehaugh Empress	**306**	12.6 c	**42**	46.5 b	**42**	9.5 a	**42**	5.7 b	**575**	1.03 c	**42**	5.4 b	**42**	47.5 a
FRIEDENHEIM (INTERMEDIATE)															
No	**Genotype**		**Yield**		**Juice**		**Peel**		**TSS**		**Acid**		**Ratio**		**Rag strength**
	Scion Key	No	(Kg/tree)	**No**	%	**No**	mm	**No**	%	**No**	%	**No**	%	**No**	%
231	Ruby Blush	**1058**	17.7 a	**1179**	49.4 a	**1179**	7.2 d	**1179**	7.2 a	**1179**	1.52 a	**231**	5.0 a	**1176**	51.4 b
1053	Marsh	**1069**	16.9 a	**1058**	48.3 ab	**1058**	7.6 d	**1058**	6.8 ab	**1176**	1.45 ab	**1058**	4.8 a	**1069**	51.7 b
1057	Marsh (Control)	**1053**	16.6 a	**231**	47.6 ab	**231**	8.1 bc	**231**	6.6 ab	**1058**	1.40 bc	**1179**	4.7 a	**1058**	52.4 b
1058	Redblush	**1057**	13.4 a	**1176**	46.4 bc	**1069**	8.7 bc	**1176**	6.5 bc	**1069**	1.37 bc	**1069**	4.7 a	**1053**	53.0 b
1069	Nartia	**1179**	12.9 a	**1069**	45.1 de	**1176**	8.8 bc	**1069**	6.3 bc	**231**	1.34 bc	**1053**	4.5 a	**1057**	53.1 b
1176	Marsh	**1176**	10.5 a	**1053**	43.1 de	**1057**	9.7 ab	**1053**	5.9 c	**1057**	1.33 bc	**1176**	4.5 a	**231**	53.7 b
1179	Star Ruby	**231**	1.0 a	**1057**	42.4 e	**1053**	9.9 a	**1057**	5.8 c	**1053**	1.30 c	**1057**	4.4 a	**1179**	57.1 a
	Rootstock Key														
575	Volckameriana	**608**	18.2 a	**306**	48.9 a	**306**	7.7 c	**306**	7.3 a	**306**	1.52 a	**608**	5.1 a	**306**	51.0 b
42	Van Staden	**575**	14.6 a	**608**	48.5 a	**608**	8.1 c	**608**	7.0 a	**608**	1.37 b	**306**	4.8 a	**42**	53.6 a
608	Carrizo Citrange	**306**	13.6 a	**575**	43.4 b	**575**	8.9 b	**575**	5.9 b	**575**	1.33 b	**575**	4.4 b	**575**	53.9 a
306	Rosehaugh Empress	**42**	8.6 b	**42**	42.4 b	**42**	9.9 a	**42**	5.5 b	**42**	1.32 b	**42**	4.2 b	**608**	53.9 a
MESSINA (DRY HOT)															
No	**Genotype**		**Yield**	**Juice**		**Peel**		**TSS**		**Acid**		**Ratio**		**Rag strength**	
	Scion Key	No	(Kg/tree)	**No**	%	**No**	mm	**No**	%	**No**	%	**No**	%	**No**	%
231	Ruby Blush	**1069**	25.1 a	**231**	43.6 a	**1057**	7.9 b	**1179**	11.1 a	**1053**	1.80 a	**1058**	8.8 a	**1058**	72.2 a
1053	Marsh	**1053**	24.0 a	**1058**	41.4 a	**1058**	8.2 ab	**1057**	10.6 ab	**1179**	1.40 a	**231**	8.7 a	**231**	72.9 a
1057	Marsh (Control)	**1176**	22.2 a	**1053**	40.9 a	**1179**	8.3 ab	**1053**	10.4 ab	**1057**	1.30 a	**1057**	8.2 a	**1176**	74.5 a
1058	Redblush	**1057**	18.0 ab	**1176**	40.3 a	**231**	8.8 ab	**1176**	10.1 bc	**1176**	1.30 a	**1069**	8.2 a	**1069**	74.5 a
1069	Nartia	**1058**	15.6 ab	**1057**	38.3 a	**1176**	8.9 ab	**1058**	10.1 bc	**1069**	1.20 a	**1176**	8.2 a	**1053**	74.6 a
1176	Marsh	**1179**	11.5 b	**1069**	37.5 a	**1053**	8.9 ab	**1069**	10.0 bc	**1058**	1.20 a	**1053**	8.1 a	**1057**	75.2 a
1179	Star Ruby	**231**	8.6 b	**1179**	36.9 a	**1069**	9.2 a	**231**	9.3 c	**231**	1.10 a	**1179**	8.0 a	**1179**	75.6 a
	Rootstock Key														
575	Volckameriana	**575**	21.9 a	**575**	40.7 a	**608**	8.2 a	**306**	10.8 a	**608**	1.70 a	**608**	8.7 a	**608**	73.8 a
42	Van Staden	**42**	20.9 a	**608**	39.8 a	**575**	8.4 a	**608**	10.5 ab	**306**	1.50 a	**575**	8.6 a	**42**	74.1 a
608	Carrizo Citrange	**306**	15.7 ab	**42**	39.2 a	**306**	8.5 a	**575**	10.1 ab	**42**	1.30 a	**42**	8.1 ab	**575**	74.3 a
306	Rosehaugh Empress	**608**	10.1 b	**306**	39.1 a	**42**	8.9 a	**42**	10.0 b	**575**	1.20 a	**306**	7.4 b	**306**	76.1 a

Values per trait and locality followed by the same alphabetically letter did not differ significantly at P≤0.05.

Table 3.9 Average means over scions and rootstocks for yield and quality of grapefruit cultivars in combination with four rootstocks harvested independently at Malalane, Friedenheim and Messina from four-year-old trees

MALALANE (HOT HUMID)							
	Yield	**Juice**	**Peel**	**TSS**	**Acid**	**Ratio**	**Rag strength**
	kg.tree^{-1}	**%**	**mm**	**%**	**%**		
Trial mean (x)	30.6	48.4	8.4	6.3	1.09	5.8	44.7
Coefficient of variance %	39.1	5.8	11.3	5.9	5.9	7.3	11.8
P Scion	0.0020***	0.0063***	0.0148*	0.0002***	0.0068***	0.0001***	0.0226*
P Rootstock	0.0001***	0.0024***	0.0001***	0.0001***	0.0001***	0.0001***	0.0013***
P Scion*Rootstock	0.0677 NS	0.7551 NS	0.5675 NS	0.0325*	0.1643 NS	0.0589 NS	0.5251 NS
FRIEDENHEIM (INTERMEDIATE)							
	Yield	**Juice**	**Peel**	**TSS**	**Acid**	**Ratio**	**Rag strength**
	kg.tree^{-1}	**%**	**mm**	**%**	**%**		
Trial mean (x)	13.8	45.8	8.7	6.4	1.38	4.6	53.1
Coefficient of variance %	55.8	5.5	12.7	12.5	8.7	11.6	6.8
P Scion	0.0196*	0.0001***	0.0001***	0.0001***	0.0014***	0.1710 NS	0.0018***
P Rootstock	0.0001***	0.0001***	0.0001***	0.0001***	0.0002***	0.0001***	0.0001***
P Scion*Rootstock	0.0332*	0.3373 NS	0.5430 NS	0.7406 NS	0.4284 NS	0.2643 NS	0.2643 NS
MESSINA (DRY HOT)							
	Yield	**Juice**	**Peel**	**TSS**	**Acid**	**Ratio**	**Rag strength**
	Yield (kg/tree)	**%**	**mm**	**%**	**%**		
Trial mean (x)	18.3	39.9	8.5	10.2	1.4	8.1	74.3
Coefficient of variance %	48.6	16.4	10.3	7.1	70.7	14.8	3.6
P Scion	0.0001***	0.1203 NS	0.0159*	0.0051***	0.6045 NS	0.7875 NS	0.2116 NS
P Rootstock	0.0001***	0.6807 NS	0.0583 NS	0.2173 NS	0.6717 NS	0.0523 NS	0.3645 NS
P Scion*Rootstock	0.0918 NS	0.0733 NS	0.0691 NS	0.0770 NS	0.3271 NS	0.5324 NS	0.7366 NS

P≤0.05, *** P≤0.01 and NS=Non significant

3.3.4 Comparing data per group in one locality over five years with Valencia as an example

The analysed data for one locality over five years (Table 3.10) confirms the trends that were eminent after only three years. All the terms and interactions effects were significant except the interaction effect of rootstock x scion for juice which was non-significant.

Table 3.10 Mean squares for yield and fruit quality traits of Valencia selections budded on different rootstocks over five years at Malalane)

		Yield		Mass		Juice		Peel		TSS		Acid		Ratio	
Source	**Df**	Ms	**P**	Ms	**P**	Ms	**P**	Ms	**P**	Ms	**P**	Ms	**P**	**Ms**	**P**
Block	**3**	2695.02	**0.187**	912.90	**0.120**	34.64	**0.114**	0.16	**0.435**	1.48	**0.123**	0.05	**0.005**	4.67	**0.031**
Scion	**8**	24281.27	**<.0001**	16674.17	**<.0001**	77.37	**0.000**	0.55	**0.004**	9.17	**<.0001**	2.50	**<.0001**	187.50	**<.0001**
Rootstock	**3**	62164.96	**<.0001**	1869.21	**0.009**	373.05	**<.0001**	5.98	**<.0001**	29.75	**<.0001**	0.93	**<.0001**	41.91	**<.0001**
RootstockxScion	**21**	2891.38	**0.038**	932.99	**0.012**	13.43	**0.722**	0.37	**0.008**	3.26	**<.0001**	0.11	**<.0001**	4.38	**0.000**
Block(S*R)	**84**	1647.99		455.88		16.97		0.17		0.75		0.02		1.51	
Year	**4**	127625.76	**<.0001**	45691.73	**<.0001**	1498.12	**<.0001**	3.53	**<.0001**	56.37	**<.0001**	0.56	**<.0001**	56.07	**<.0001**
YearxScion	**32**	2070.71	**<.0001**	2192.56	**<.0001**	30.11	**<.0001**	0.46	**<.0001**	2.80	**<.0001**	0.03	**<.0001**	3.88	**<.0001**
YearxRootstock	**12**	8052.81	**<.0001**	673.05	**0.016**	26.73	**0.005**	0.55	**<.0001**	11.30	**<.0001**	0.15	**<.0001**	4.37	**<.0001**
YxRSxS	**84**	1148.86	**<.0001**	829.49	**<.0001**	16.93	**0.005**	0.16	**0.024**	0.99	**<.0001**	0.02	**<.0001**	1.74	**0.001**

Df = Degrees of freedom and Ms = Mean squares

3.4 DISCUSSION

3.4.1 Yield and quality data of Valencia selections in one locality over three years

Results as represented in Table 3.2 to 3.7 represents five years of a total of 10 years of data taken at Malalane on the seven Valencia genotypes and only one of five citrus types. The dialogue with regard to the five years (not presented here) as well as the other four citrus types followed the same trend. Significant differences amongst scion genotypes ($P \leq 0.05$ and some even $P \leq 0.01$) were evident on an annual basis and the same applied to rootstock genotypes. However, it is very difficult to identify truly superior cultivars from the available data tables. Over the three years the industry standard Valencia cultivar, 1043, and new selection 1044 were constantly lower performing than the other cultivars. The rest of the cultivars did not differ significantly from each other in any of the years except for 1055 that seemed to drop out by being significantly lower performing than 1056 when the trees reached six years of age. Although 1056 always ended up being the top producer, it did not differ statistically from the other cultivars tested.

Alternate bearing was already evident from Table 3.2 to 3.7 with yields for six-year-old trees being lower than that of the five-year-old trees for all the genotypes except 1043. One way to compensate for this, in determining the most suitable selection after ten years of evaluation, could be comparing cumulative yields. With regard to quality, traits there appeared to be a rank crossover GEI but without the appropriate analysis the nature and magnitude is unknown.

With regard to rootstocks, performance on 575 with regard to yield in all three years was significantly better than on any of the other three rootstocks which displayed ranking differences when the trees were five and six years old. Although the attribute of rootstocks to the variance in quality attributes was significant in the majority of the attributes over the three years, no clear pattern could be detected for the selection of the best suitable rootstock for the area.

3.4.2 Discussion on comparing data tables of a citrus type (grapefruit) amongst three localities for a single year using grapefruits as an example

Data of four-year-old grapefruit selections were included for three localities in this comparison (single year data). The data clearly implied GEI with regard to yield with 231 the highest-ranking cultivar at Malalane but the lowest ranking cultivar at Messina. No cultivar recommendation can be done on only one years' data but at Malalane 1179 and 231 seemed to be superior in all aspects except rag strength where they were the worst performers. At Friedenheim, 1179 was also the favourite cultivar after only one years' data for all attributes again excluding rag strength. At Messina, the control cultivar 1057 was a winning cultivar.

The effect of the different rootstocks was also more profound at Malalane than at Friedenheim and Messina with 575 imparting almost triple the yield at Malalane than at any of the other localities. The data again implied that there was GEI but again the data analysis did not indicate the nature or magnitude of these interactions. The data also noted scion x rootstock interaction but no deduction can be made with regard to the specific genotype x genotype interaction.

3.4.3 Discussion on annual data analysis of quality per single locality using Valencia data at Malalane

The ANOVA done over five years incorporates years as an environment instead of relying on annual differences in means between genotypes. Data analysis (Table 3.10) indicated that all traits were significantly influenced by both the scion and the rootstocks individually. The data also indicated a significant interaction of the rootstock with the scion except for the juice, which was non-significant.

However, in analysis of the years as separate events it was found that some of the terms were non-significant for a specific year. It would thus appear as though some years are more conducive of the specific traits than other years. The data analysed over five years did prove that there was significant GEI.

3.5 CONCLUSIONS

Mega-environments have been defined in different ways, but the main idea is that it is a broad, not necessarily contiguous area, defined by a similar factor such as biotic and abiotic stress, cropping system requirements, consumer preference, or even volume of production. A mega-environment is also defined as a portion of a crops' growing region with a homogenous environment that causes some genotypes to perform similarly (Gauch and Zobel, 1996,1997) and by Yan and Rajcan (2002) as a group of localities that consistently share the same best cultivar(s). Per this definition, the climatic zones as defined by Barry (1996) and familiar to the South African citrus industry act as predefined mega environments.

Each of the five localities was deemed a mega-environment with the aim to test the new cultivars against an industry standard. The aim to identify genotypes with an improved performance over a standard control cultivar was reached annually with regard to most of the traits if only genotype (G) as scion and rootstocks was taken into account. However, although GEI was implicated in Table 3.10 the univariate method was not able to specify the magnitude or detail of this interaction at the level of specific scion-rootstock combinations. According to Shaffi and Price (1998) the most commonly used statistical technique for analysing GEI is the two-way cross-classification analysis of variance (ANOVA) and that while this technique is useful in identifying and testing sources of variability, it does not express or describe patterns of the underlying interaction.

Regarding the Valencia group at Malalane the conclusion was that all of the new cultivars were better than the standard Valencia cultivar (1043) and could be recommended and that the best rootstock for yield was 575. The same applied to quality aspects where the standard Valencia cultivar (1043) almost constantly displayed the lowest quality. However, rank changes over years made the discrimination amongst the other cultivars difficult. The best rootstock with regard to quality was 608, which performed on par with 306. The latter was later discarded due to other horticultural reasons such as ease of propagation and disease resistance.

The comparison of data per group (grapefruit) over three localities in a single year using grapefruits as an example clearly implied GEI with regard to yield with 231 the highest-ranking cultivar at Malalane but the lowest ranking cultivar at Messina. GEI was also evident with regard to rootstocks at different localities as the effect of rootstocks was more profound at Malalane than at Friedenheim and Messina.

GEI proved to be complex as illustrated by ranking changes between analysis from one year to another and the high significance of GEI in the ANOVA over five years where E was years. This was confirmed by the data in Tables 3.8 and 3.9 comparing data of the same genotypes across physical localities. GEI is usually defined by pure environmental factors such as climate and soil but here the problem is even more challenging. In the case of a tree crop where the manifestation of the yield and quality attributes is also influenced by the rootstock, the rootstock has a G, GEI and E component in itself.

According to Yan and Tinker (2006) the objectives of data analysis within a single mega-environment should identify genotypes with both high performance and high stability and should identify test environments that are both discriminating and representative. In addition, whenever there is significant GE, potential causes of GE should be explored and exploited.

It is thus evident that statistical tests such as ANOVA and t-tests are not sufficient to unravel the GEI and its subsequent interactions with the environment. An ordinary ANOVA allows for sufficient description of main effects based on the additive nature of the ordinary ANOVA. However, interactions are represented by the residual from the additive model, which is nonadditive and requires other techniques to identify the interaction relationships (Shaffi and Price, 1998). Several statistical methods have been proposed for increasing the chance of exploiting positive GEI and supporting breeding programme decisions in variety/cultivar selection and recommendation for target set of environments. Amongst these are the additive main effects and multiplicative interactions (AMMI) and genotype plus genotype-by-environment interaction (G+GE) models that effectively capture the additive (linear) and multiplicative (bilinear) components of GEI and provide meaningful interpretation of MET data in breeding programs.

It is therefore recommended that these models be investigated with the available data for future applicability in a fruit-breeding programme.

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Chapter 4

RELEVANCE OF AMMI FOR INVESTIGATION GEI PERTAINING TO YIELD AMONGST CITRUS SCIONS, ROOTSTOCKS AND ENVIRONMENTS

Abstract

Cultivars with high and stable genetic potential for production and quality is the main goal of a fruit-breeding programme and are assessed in multi-environment trials due to the influence of rootstocks as well as climate on yield in citrus. These environments can be either artificial such as various levels of fertiliser or it can be natural such as different production seasons or climate zones. In a crop where grafting is essential, the manifestation of the scion's genotype is dependent on the rootstock on which it is grafted as well as the environment in which the scion-rootstock combination (stion) is grown. The problem with grafted trees is that part of what is measured in these trials is the rootstock's reaction to the environment as well as the rootstock's interaction with the scion and vice versa, which constitutes complex genotype x environment interactions (GEI). The aim of this investigation was to explore the relevance of the additive main effects and multiplicative interaction (AMMI) model to investigate GEI amongst citrus scions, rootstocks and environments in the traditional citrus scion Phase 2 trial layout. Superior genotypes selected from the ARC-ITSC breeding programme as well as newly imported cultivars from other breeding programmes in the world are annually included in replicated field (Phase II) trials and evaluated against a known cultivar control. Five scion evaluation trials were chosen for this study since they were planted at the same time, grafted on the same four rootstocks and evaluated for five years. Scion species encompassed grapefruit, midseason oranges, Valencia oranges, mandarins (C. reticulata – Early) and Ellendales (C. reticulata – Late). Scions were grafted onto four different rootstocks namely Van Stadens rough lemon (C. jambhiri, Tenaka), Volckamer lemon (Citrus volkameriana V. Ten. & Pasq.), Empress Rosehaugh (C. reticulata Swingle) and Carrizo citrange (Poncirus trifoliate x C. sinensis). Regarding the question pertaining to whether there is any GEI with regard to rootstock grafted to very different citrus scion types, it was found that GEI was significant and that rootstock evaluation should be specific to each mega-environment (citrus scion type). It was also determined that stion GEI can successfully be separated into a scion GEI and a rootstock GEI for yield per mega-environment (citrus scion type). With regard to variation within a group it was found that there was interaction amongst rootstocks and scions, except for mandarins. The interaction of the rootstocks and scions with environment (year) and the impact thereof on the stion were differential and substantial. Interpretation of the AMMI results was difficult, as the AMMI model does not make provision for a quantitative stability measure. Such a measure is essential in order to quantify and rank genotypes according to their yield stability.

4.1 INTRODUCTION

Development of cultivars with high and stable genetic potential for production and quality is the main goal of a fruit-breeding programme. However, this is also one of the main challenges of a fruit-breeding programme as almost all of these traits are complex due to gene action and interaction with the environment, that is, different reactions of diverse genotypes to the same environment or different reactions of the same genotype to dissimilar or changing environmental conditions. Genotype phenotypic expression of a trait in a specific environment consists of genotypic main effects, environment effects and the interaction of the two. A major experimental effort in fruit cultivar performance and plant breeding research therefore consists of multi-locality trials. In crops such as wheat the objectives of these trials are:

- to assess the success of yield prediction
- to group sites for evaluation, and
- to interpret GEI.

In fruit farming, such as with trees and vines, choice of cultivar is an expensive and long-term investment. New fruit cultivars are pursued by producers in an effort to enhance their export market share. An essential question to be answered before a new cultivar is adopted is the long-term suitability of the cultivar to the farmers' environment. When differential responses of genotypes to environments, in other word GEI is present, effects of genotypes and environments are statistically non-additive. It is thus perceivable that GEI may lead to different rank orders of genotypes in different environments, a difference in scale among environments, or a combination of these two situations, which complicates cultivar recommendations.

Literature has shown that there is a definite influence of rootstock on scion and a definite influence of climate on stion (Koepke and Dhingra, 2013). In a crop where grafting is essential, the manifestation of the scion's genotype is dependent on the rootstock on which it is grafted as well as the environment in which the scion-rootstock combination (stion) is grown. Phenotypic response is specific in relation to stionic combinations used, and new hybrids must be evaluated for these reactions (Hume, 1957). A grafted or budded plant can produce growth patterns and reactions which may be different from what would have transpired if each part of the stion was grown separately or when the scion was grafted or budded on other types of rootstocks (Figure 4.1).

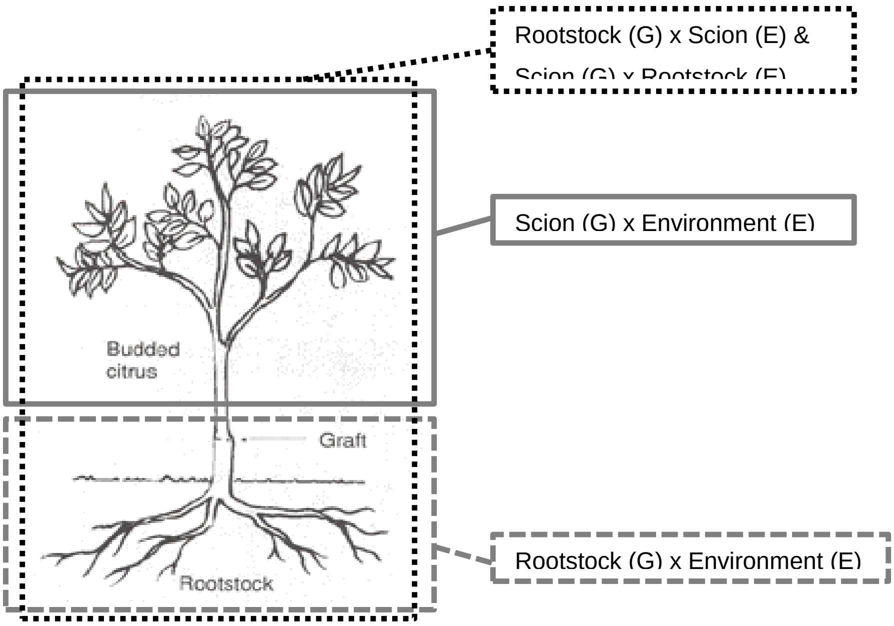

Figure 4.1 The complex genotype x environment context of a grafted tree. Environment can represent the physical environment (soil or climate) or year

It is thus not only a question of long-term suitability of the cultivar to the producers' environment which encompass cultivar suitability as well as stability but it also relates to the rootstock to be used and its' suitability and stability in the production environment. Equally important is the interaction between rootstock and scion and the impact thereof on the stion's stability. Consequently, breeders evaluate cultivar performance across many environments in what is commonly referred to as multi-environment trials (METs). The problem with grafted trees is that part of what is measured in these trials is the rootstock's reaction to the environment as well as the rootstock interaction with the scion and *vice versa*.

However, on face value it might seem impossible to separate scion (G) x environment (E) from rootstock (G) x environment (E) from that of the stion, the reason being that the scion and rootstock are interdependent on each other. The main objective of this study was thus to determine whether it is possible to systematically partition the reaction of the stion into scion and rootstock reactions.

According to Gauch (1992) a MET often (not always) consists of the same set of cultivars planted in the same year at different localities. The purpose of MET networks is to evaluate and select promising cultivars for commercial production in a target region. Yan and Tinker

(2005) stated that dividing target environments into meaningful mega-environments is the only way that complex GEI can be exploited. Gauch and Zobel (1997) defined a mega-environment as a portion of a crop species' growing region with a homogeneous environment, causing some genotypes to perform similarly and is normally identified through analysis of MET data.

For the purpose of this study, one can postulate that the target region for a new rootstock cultivar is the total component of commercial citrus scions and *vice versa*. Once the relevant contribution of scion and rootstock to the stion has been quantified in a single physical environment (i.e same climate, same soil, same production practices) the interaction of each can be determined in varying physical environments which would pertain to the farmer's problem of adopting new cultivars with regard to suitability and stability. For the purpose of this study the varying physical environments pertains to year differences.

A combined ANOVA can quantify the interactions and describe the main effects but cannot explain GEI (Odewale *et al*., 2013) as was illustrated in Chapter 3. A wide range of methods is available for analysis of G by E and can be broadly classified into four groups: analysis of components of variance, stability analysis, multivariate methods and qualitative methods.

For the purpose of MET studies, Gauch (1992) has adopted the use of AMMI analysis. Gauch and Zobel (1988) compared the performance of AMMI analysis with the ANOVA approach and regression approach and found that ANOVA fails to detect a significant interaction component and the regression model accounts for a small proportion of the interaction sum of squares (SS) only when the pattern fits a specific regression model.

The objectives of this study towards systematically partitioning the reaction of the stion into scion and rootstock reactions were to:

(i) quantify GEI with regard to rootstock grafted to different citrus scion types
(ii) separate stion GEI into a scion GEI and a rootstock GEI for yield per mega-environment (citrus scion type) and
(iii) differentiate scion (G) x environment (E) and rootstock (G) x environment (E) interaction from that of the stion.

4.2 MATERIAL AND METHODS

4.2.1 Materials

4.2.1.1 Scion material

Promising scion and rootstock selections from the ARC-ITSC breeding programme are annually included in replicated field (Phase II) trials and evaluated against a known control cultivar. Due to the diversity of the Citrus genus as explained in Chapter 2, rootstock selections were grafted for evaluation with seven commercial citrus scion types namely *C. sinensis* (three types: Navels, Midseason and Valencia), *C. limon, C. paradise*, *C. reticulate* and at the cold production area, *C. unshi* was included (Table 3.1). Phase II scion trials comprised of selections from each of the citrus types evaluated against a commercial control cultivar, budded on to one or several rootstocks, and planted in replicated field trials in multiple locations.

Five scion evaluation trials were chosen for this study since they were planted at the same time and the same rootstocks were used in all five trials and encompassed *C. sinensis* (two types: Midseason and Valencia), *C. paradise*, *C. reticulate*-early (mandarins) and *C. reticulate*-late (Ellendales). For the benefit of graphs and tables, these five citrus citrus scion types were designated repectively Mid, Val, Grf, Man, and Ell. The number of promising selections and the control cultivar per citrus group for each of the five Phase II trials that were planted at the same time, are listed in Table 4.1.

Table 4.1 Controls and number of citrus genotypes per citrus scion type included in five Phase II trials at Malalane ARC-ITSC

Trial	Citrus scion type	Number of genotypes (inclusive of the control cultivar)	Control cultivar
1	*C. sinensis* – midseason (Mid)	3	Tomango (cv. no.1047)
2	*C. sinensis* – Valencia (Val)	9	Delta (cv. no.1043)
3	*C. paradisi* – grapefruit (Grf)	9	Marsh (cv. no. 1057)
4	*C. reticulata* – early (Man)	7	Nova (cv. no. 803)
5	*C. reticulata* – late (Ell)	6	Beauty (cv. no. 228)

4.2.1.2 Rootstocks used

Scions were grafted onto four different rootstocks namely Van Stadens rough lemon (*C. jambhiri,* Tenaka)*,* Volckamer lemon (*Citrus volkameriana* V. Ten. & Pasq.), Empress Rosehaugh (*C. reticulate* Swingle*)* and Carrizo citrange (*Poncirus trifoliate* x *C. sinensis)*. For the benefit of graphs and tables, the respective cultivars numbers in the ARC-ITSC gene bank for these rootstocks namely 42, 575, 306 and 608 were used.

4.2.1.3 Localities

This study was carried out on five citrus scion types at one locality namely Malalane, being a hot humid (0.5 - 0.65 P/PET) locality as classified by (Barry, 1996) and is 327 m above sea level. Its coordinates are 25°27'49" S and 31°35'15" E 25°28'60" N and 31°34'60" E in DMS (Degrees Minutes Seconds) or -25.4833 and 31.5833 (in decimal degrees). The trial site has a very shallow Mispah soil type.

4.2.1.4 Trial layout

The trial layout was a randomised block design with four rootstocks and four trees per rootstock budded to each of the genotypes thus16 trees per genotype.

4.2.2 Methods

The methods with regard to virus cleansing, cross protection, nursery practices, production practices and evaluation of traits were similar to that of Chapter 3.

4.2.2.1 Statistical analysis

GEI for fruit production was analysed according to a classical multiplicative model or AMMI (Gauch, 1992) with two multiplicative terms. It is written as follows:

$$E[Y_{ge}] = \mu + \alpha_g + \beta_e + \sum_{n=1}^{N} (\alpha\beta)^n{}_{ge} + \epsilon_{ge}$$

where

Y_{ge} is the measured trait (e.g. yield) of genotype g in environment e production

μ is the grand mean

α_g is the genotype g mean deviation

β_e is the environment e mean deviation

$\sum_{n=1}^{N} (\alpha\beta)^n{}_{ge}$ is a sum of N interaction components for genotype g in environment e and

ϵ_{ge} is the residual

The AMMI model (Gauch, 1992) was developed to facilitate the interpretation of GEI and identify genotypes adapted to specific localities. In brief, an AMMI uses conventional ANOVA to partition variance into three components namely genotype deviations from the grand mean, environment deviations from the grand mean and GEI deviations from the grand mean. Next, multiplication effect analysis is used to partition GEI deviations into different principal component axes (PCA), which can be tested for statistical significance through an ANOVA. The AMMI model thus uses ANOVA for additive main effects (AM) and principle component analysis (PCA) for multiplicative interactions (MI) to identify patterns in the data (Crossa, 1990). Results of an AMMI are given in the form of an ANOVA table as well as visualised by means of a biplot that shows the relationships between the eigenvalues for the PCA and the means of the genotypes and environments (Kempton, 1984). AMMI analysis was constructed using GenStat 15th edition (Payne *et al*., 2012). Shapiro-Wilk test was performed to test for normality of the residuals (Shapiro and Wilk, 1965).

4.2.2.2 Procedure of partitioning the stion GEI into a scion GEI and a rootstock GEI for yield

Questions to be answered were:

Is there any GEI with regard to rootstock grafted to very different citrus scion types?

- If there is no significant GEI pattern it would mean that the target environment (*Citrus* spp) with regard to rootstock interaction, is a single mega-environment with unpredictable GEI and models addressing random sources of variation may be appropriate (Yan and Tinker, 2006).
- Significant GEI will mean that rootstock evaluation should be specific to each mega-environment (citrus scion types).

Can the stion GEI be separated into a scion GEI and a rootstock GEI for yield per mega-environment (citrus scion types)?

- In other words can the relevant contribution of the scion and rootstock to the stion be separated and quantified in a single physical environment (i.e same climate, same soil, same production practices)

Can scion (G) x environment (E) and rootstock (G) x environment interactions (I) be differentiated from that of the stion?

- In other words once the relevant contribution of the scion and rootstock to the stion have been quantified in a single physical environment (i.e. same climate, same soil, same production practices) can the interaction of each be determined in varying physical environments which would pertain to the farmer's problem of adopting new cultivars with regard to suitability and stability. In this case varying physical environments pertains to year differences.

If the data following the first question shows that there are no differential genotypic (rootstock) responses to definably diverse environments (mega-environments) in this case citrus scion types, it is perceivable that there would be no differential genotypic (rootstock) response within a citrus scion type. However, due to the highly heterogeneous nature of citrus, a differential genotypic (rootstock) response amongst the citrus scion types can also denote a differential genotypic (rootstock) response within a specific citrus scion type.

In order to determine the influence of the scion genotype on the rootstock genotype the rootstocks were budded with five very different citrus scion types as described in section 4.2.1.1. In this case, citrus scion types were considered environments (Figure 4.2).

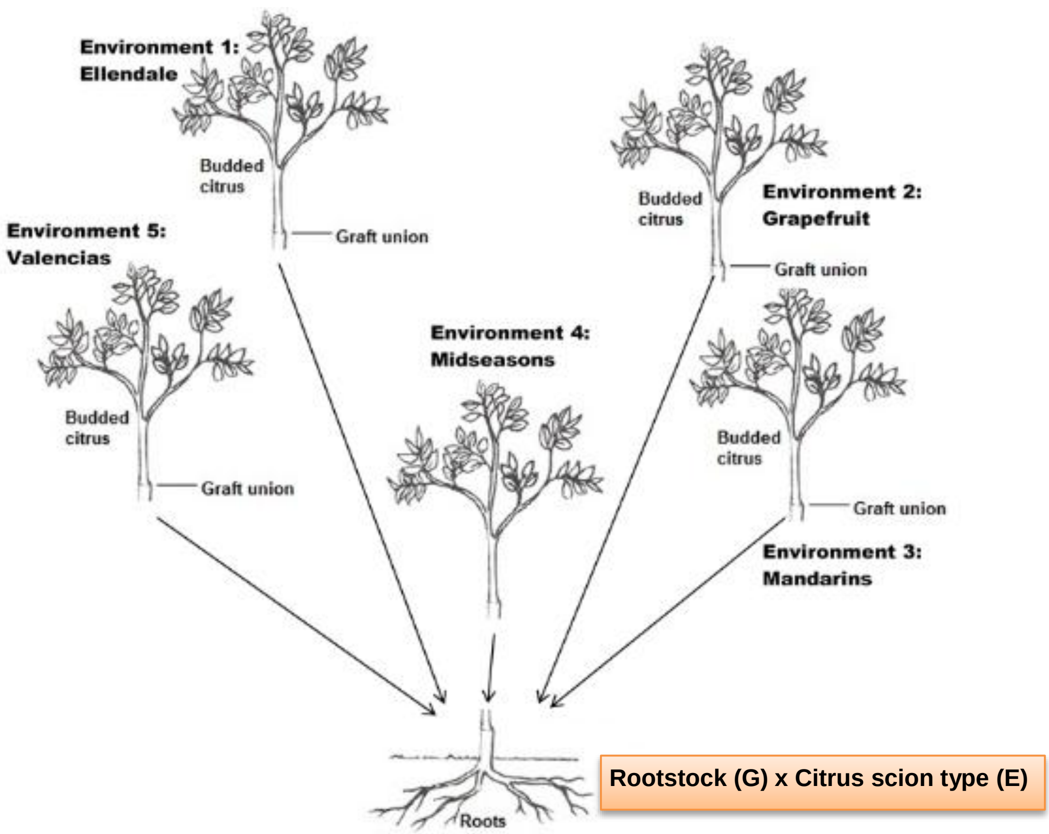

Figure 4.2 An illustration of partitioning the stion GEI by defining the rootstock (G) in different environments (E) (citrus scion types)

If the data identify a meaningful GEI it will indicate that there is a differential genotypic rootstock and scion interaction. In a standard ANOVA procedure a series of (G) genotypes in (E) environments would result in an interaction sum of squares (SS) with (G-1)(E-1) degrees of freedom (df) which could be difficult to interpret (Smith, 1992) as was illustrated in Chapter 3.

The procedure for questions 2 and 3 entails that a differential genotypic (rootstock) response within a specific citrus scion type must be determined. Figure 4.3 illustrates the partitioning of the stion GEI by testing the scion (G) on different rootstocks (E) as well as years (E) while Figure 4.4 illustrates the partitioning of the stion GEI by testing the rootstock (G) with different scions (E) as well as years (E)

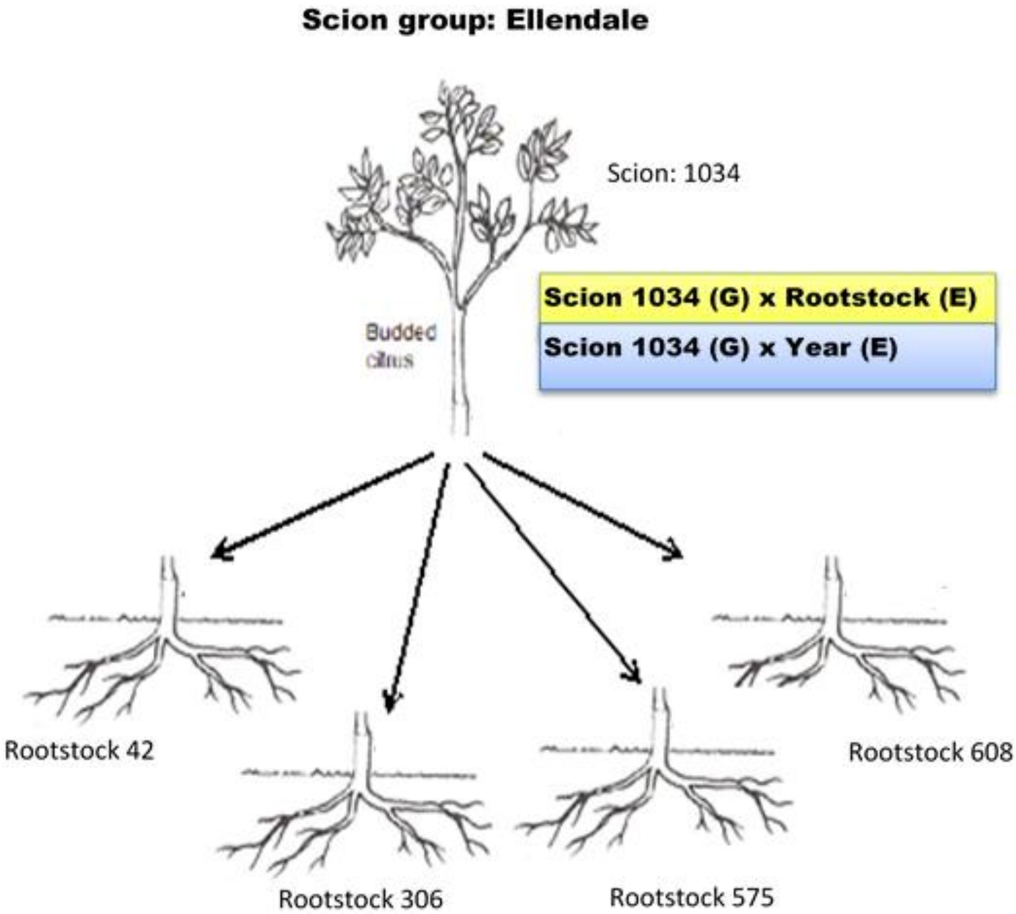

Figure 4.3 An illustration of partitioning stion GEI by defining the scion (G) in different environments (rootstocks). Thus rootstock effect on scion and year effects on scion

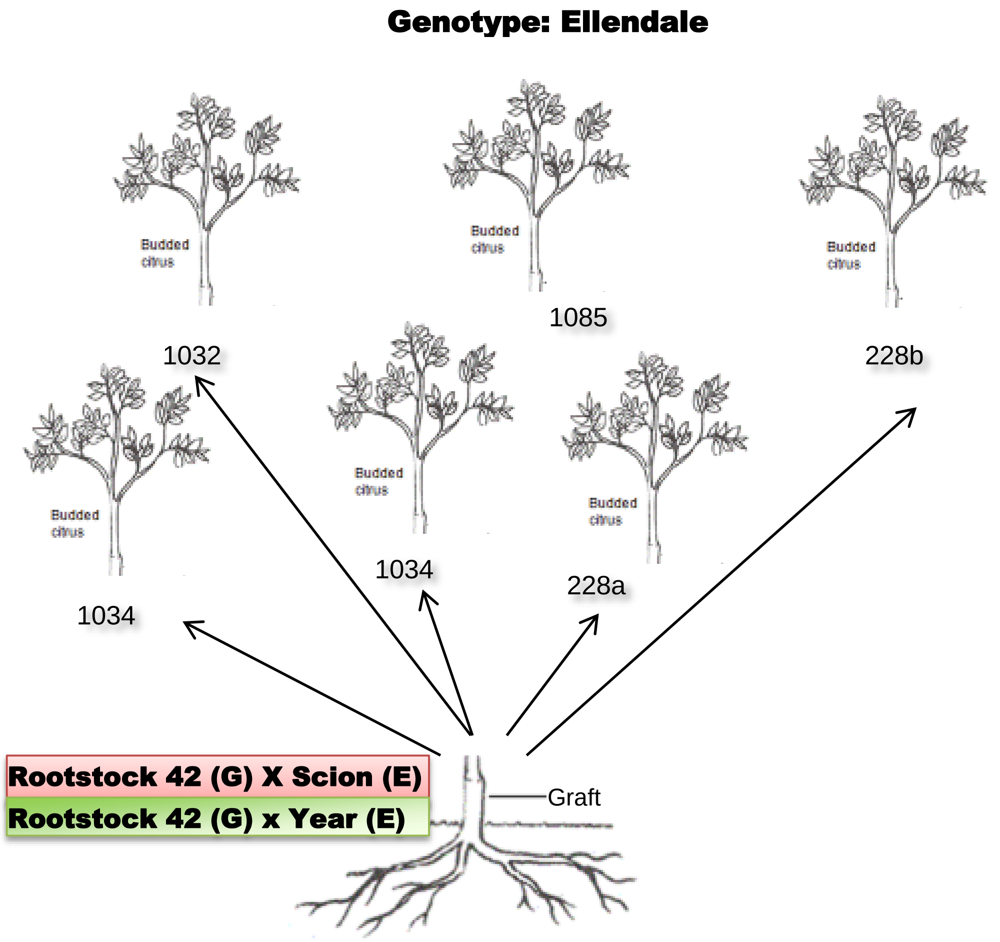

Figure 4.4 An illustration of partitioning stion GEI by defining the rootstock (G) in different environments (scion) thus scion effect on rootstock and year effects on rootstock

With the AMMI model, each environment is characterised with an environment 'score' for each component. Correspondingly, genotype performance is characterised for each component with a genotype score. Experimental results can now be explained via the weighted sum of products of such scores and cultivar response in differing environments can even be predicted via the model.

A differentiation of different mega-environments reflects repeatable crossover GEI. The presence of different mega-environments is indicated only if (1) there is GEI; (2) the GEI is large enough to lead to crossovers; and (3) the environment grouping due to the crossover GEI is repeatable over years. Thus, study of GEI over a span of years is essential to the investigation of mega-environments. Both genotypic main effect (G) and GEI must be jointly examined using multiple year data.

4.3 RESULTS

4.3.1 Defining rootstock (G) with regard to citrus scion type effect on rootstock

When field trials are carried out in different agroecological conditions, usually 80% of variation is caused by environment, while genotype and GEI usually explain 10% of variation each (Yan, 2001).

The AMMI ANOVA for yield of four rootstocks genotypes evaluated with five different citrus scion genotype (Figure 4.2) is presented in Table 4.2 and showed significant effects for genotype, environment and GEI with regard to yield for all five citrus scion types (trials).

Table 4.2 AMMI analysis of variance for yield of four rootstocks genotypes (G) evaluated with five different citrus scion types (E) over five years

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained: Total variance	Treatment variance	Interaction components
Total	79	118064	1494				
Treatments	19	115052	6055	0			
Genotypes (G) (rootstocks)	3	9690	3230	0.00	**8.21**	8.42	
Environments (E) (citrus scion types)	4	97759	24440	0.00	**82.80**	84.97	
Block	15	893	60	0.26	0.76		
Interactions	**12**	**7602**	**634**	**0.00**	**6.44**	6.61	
IPCA	**6**	**5598**	**933**	**0.00**			**73.64**
Residuals	**6**	**2004**	**334**	**0.00**			26.36
Error	45	2119	47		1.79		
					100.00	100.00	100.00

The model revealed that differences between the environments (citrus scion types) accounted for 84.97% of the treatment SS. Genotypes (rootstocks) and GEI also contributed significantly to variation at 8.42 and 6.61% respectively. The SS for the first principal component axis (IPCA) was significant at P≤0.05 and captured 73.64% of the interaction SS in 50% of the df. The other 26.36% of the variation (within 50% of the interaction df) was left in the residual.

A large SS for environments (in this case citrus scion types) indicated that the environments (citrus scion types) were diverse, with large differences among environmental means causing most of the variation in yield. This was to be expected, as the environments, namely the citrus scion types, are very diverse by nature, ranging from a small mandarin to a large grapefruit. Mean yields for the citrus scion types varied from

36.87 kg tree^{-1} to 130.33 kg tree^{-1}. Cultivar means, ranking of the four rootstocks per environment and first IPCA component scores of the rootstock cultivars are presented in Table 4.3. From this table it can be seen that rootstock 575 was prominent in four of the five environments (citrus scion types) and second in the fifth.

Table 4.3 Mean yield (kg tree^{-1}) ranking (1-4) of the citrus rootstock genotypes (G) per environment (E) (citrus scion types) in one locality according to the AMMI model

Citrus scion types (E)				Ammi selections (rootstocks) per citrus scion types (e)			
Environment (Env.)	Env. mean	IPCA 1 Score	IPCA 1 Score	1	2	3	4
Grapefruit (Grf)	130.33	-4.12	-1.06	575	608	42	306
Valencia (Val)	82.54	-2.10	2.13	575	608	42	306
Ellendale (Ell)	55.76	1.24	1.46	575	42	608	306
Midseason (Mid)	36.87	1.46	-3.47	575	42	608	306
Mandarins (Man)	38.06	3.52	0.95	42	575	306	608

According to Fox *et al*. (1990), a genotype usually found in the top third of entries across environments can be considered relatively well adapted for the trait investigated. However, in this case the magnitude of the genotype SS was 1.27 times larger than that of the GEI, but contribution to total variance by both genotype and GEI was also highly significant and within the same range. According to Farshadfar *et al*. (2011), the larger the IPCA (interaction principal component analysis) scores, either negative or positive, the more specifically adapted a genotype is to certain environments and smaller IPCA scores indicate a more stable genotype across environments. The AMMI score for GEI for grapefruit was -4.12 and for mandarin 3.52 in comparison to Ellendale and the midseason citrus scion types of 1.24and 1.46 respectively. GEI was thus large enough to cause crossovers, indicating that there were differences in genotypic (rootstock) response over environment (citrus scion type) warranting further investigation.

4.3.2 Defining scion (G) with regard to environment (E)

4.3.2.1 Defining scion (G) with regard to rootstock effect on scion within a citrus scion type (Group 1: Ellendale mandarin)

There was a significant interaction amongst rootstocks and citrus scion types (Table 4.2) in this trial. To determine rootstock effect on scion, the variation due to citrus scion type effect was removed and genotypes within a citrus scion type were thus used to determine the effect of rootstocks on the scion (Figure 4.3). AMMI ANOVA for yield of six Ellendale selections evaluated on four different rootstocks is presented in Table 4.4 and shows significant effects for genotype (G), environment (E) and GEI with regard to yield on all four rootstocks.

Table 4.4 AMMI analysis of variance for yield of six Ellendale selections (G) within the Ellendale citrus scion type of genotypes evaluated on different rootstocks (E) over five years

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained: Total Variance	Treatment variance	Interaction components
Total	95	31960	336.4				
Treatment	23	23875	1038	0.00			
Environment (E) (rootstocks)	3	5144	1714.6	0.00	**16.10**	**21.55**	
Block	12	1283	106.9	0.51	4.01		
Genotype (G) (Within a citrus scion type)	5	11552	2310.5	0.00	**36.15**	**48.39**	
Interaction	15	7179	478.6	0.00	**22.46**	**30.07**	
IPCA 1	7	6617	945.2	0.00			**92.17**
IPCA 2	5	527	105.4	0.47			**7.34**
Residual	3	35	11.8	0.96			**0.49**
Error	60	6802	113.4		21.28		
					100.00	100.00	100.00

The model revealed that differences between the environments (rootstocks) accounted for 21.55% of the treatment SS. Ellendale selections (G) and GEI contributed significantly at 48.39 and 30.07% respectively. The SS for the first principal component axis (IPCA1) was significant at P≤0.05 and captured 92.17% of the interaction SS in 47% of the df. The other 7.83% of the variation (within 53% of the interaction df) was left in the IPCA2 and residual combined and was not significant.

Cultivar means, ranking of the six Ellendale selections per environment (rootstock) and first IPCA component scores of the Ellendale selections are presented in Table 4.5. Mean yields of the four rootstocks varied from 48.80 kg tree^{-1} to 66.83 kg tree^{-1}.

Table 4.5 Mean yield (kg tree^{-1}) ranking (1-4), of Ellendale selections (G) per rootstock (E) in one locality, according to the AMMI model

Rootstocks (E) Environment (Env)	Env. mean	Score	AMMI (Ellendale) selections (G) per rootstock (E): 1	2	3	4
42	58.5	-4.49	1085	1032	1034	228a
575	66.83	-0.24	1085	1032	1034	228a
306	48.8	0.22	1085	1032	1034	228a
608	49.75	4.51	1032	1034	228a	1033

A large SS for rootstocks (E) would have indicated that the environments (rootstocks) were diverse, with large differences among environmental means causing most of the variation in yield. In this case, the magnitude of the genotype SS was 1.6 times larger than that of the GEI, which would indicate that there were little differences in genotypic response over rootstocks (E). However, the contribution of GEI in explaining the total variance was significant as well as substantial enough to result in crossovers among rootstocks (E).

4.3.2.2 Scion (G) and environment interaction (Group 1: Ellendale mandarin)

To determine the year effect on the scion, the variation due to group effect were removed and genotypes within a group were thus used to determine the effect of the environment (years) on the scion (Figure 4.3). AMMI ANOVA for yield of six Ellendale selections evaluated in five consecutive years is presented in Table 4.6 and shows significant effects for genotype, environment and GEI with regard to yield on all years.

Table 4.6 AMMI analysis of variance for yield of six Ellendale selections (G) within the Ellendale citrus scion type evaluated for five consecutive years (E) at one locality

					% Variance explained		
Source of variance	df	SS	MS	Probability (P≤0.05)	Total Variance	Treatment variance	Interaction components
Total	119	177893	1495				
Treatment	29	169671	5851	0.00			
Environment (years)	4	141764	35441	0.00	**79.69**	**83.55**	
Block	15	457	30	1.00	0.26		
Genotype (Ellendale selections)	5	14440	2888	0.00	**8.12**	**8.51**	
Interaction	**20**	**13467**	**673**	**0.00**	**7.57**	**7.94**	
IPCA 1	**8**	**9929**	**1241**	**0.00**			**73.73**
IPCA 2	**6**	**2918**	**486**	**0.00**			21.67
Residual	6	620	103	0.43			4.60
Error	75	7766	104		4.37		
					100.00	100.00	100.00

The model revealed that differences between the production years (E) accounted for 83.55% of the treatment SS. Ellendele selections (G) and GEI also contributed significantly at 8.51 and 7.947% respectively. The SS for the first principal component axis (IPCA1) was significant at P≤0.05 and captured 73.73% of the interaction SS in 40% of the df. The SS for the second principal component axis (IPCA2) was significant at P≤0.05 and captured 21.67% of the interaction SS in 30% of the df. The other 4.60% of the variation was left in the residual and was not significant.

A large SS for production years (E) indicated that the environments (production years) were diverse, with large differences among the environmental means causing most of the variation in yield. The magnitude of the GEI SS was almost equal to that of the Ellendael selections (G).

Cultivar means, ranking of the first four of the six Ellendale selections (G) per rootstock (E) and first IPCA component scores of the Ellendale selections are presented in Table 4.7. Mean yields of the five environments varied from 107.62 kg tree^{-1} to 11.28 kg tree^{-1}.

Table 4.7 Mean yield (kg tree^{-1}) ranking (1-4) of Ellendale selections (G) per years (E) at one locality, according to the AMMI model

Years (E)			AMMI Ellendale selections (G) per year (E)			
Environment (Env.)	Env. mean	Score	1	2	3	4
Y5	107.62	-5.48	1085	1032	1034	228a
Y1	25.19	-0.65	1085	1032	1034	228a
Y4	65.53	-0.08	1085	1034	1032	228a
Y3	70.21	2.88	1032	228a	1034	1085
Y2	11.28	3.33	228a	1034	1033	1085

4.3.3 Defining rootstock (G) with regard to environment (E)

4.3.3.1 Defining rootstock (G) with regard to scion effect (Citrus scion type 1: Ellendale mandarin) on rootstock (E)

To determine the scion effect on the rootstock, the diversity due to citrus scion type (group effect) was removed and genotypes within one citrus scion type were thus used to determine its effect on the rootstock (Figure 4.4). AMMI ANOVA for yield of four rootstock genotypes (G) with six Ellendale selections evaluated for five consecutive years is presented in Table 4.8 and shows significant effects for genotype, environment and GEI with regard to yield on all years.

The model revealed that differences between the Ellendale selections (E) accounted for 48.39% of the treatment SS. Rootstocks (G) and GEI contributed significantly at 21.55 and 30.07% respectively. The SS for the first principal component axis (IPCA1) was significant at $P \leq 0.05$ and captured 92.17% of the interaction SS in 47% of the df. The other 7.83% of the variation (within 53% of the interaction df) was left in the IPCA2 and residual combined and was not significant.

Table 4.8 AMMI analysis of variance for yield of four rootstocks (G) evaluated with six Ellendale selections (E) within the Ellendale group

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained: Total Variance	Treatment variance	Interaction components
Total	95	31960	336.4				
Treatments	23	23875	1038	0.00			
Environments	5	11552	2310.5	0.00	**36.15**		
(Ellendale Selections)						**48.39**	
Block	18	2327	129.3	0.28	7.28		
Genotypes (rootstocks)	3	5144	1714.6	0.00	**16.10**	**21.55**	
Interactions	**15**	7179	478.6	0.00	**22.46**	**30.07**	
IPCA 1	**7**	6617	945.2	0.00			**92.17**
IPCA 2	**5**	527	105.4	0.43			7.34
Residuals	3	35	11.8	0.95			0.49
Error	54	5758	106.6		18.02		
					100.00	**100.00**	100.00

The large SS for the Ellendale selections (E) indicated that the environments (selections) were diverse, with large differences among itsl means causing most of the variation in yield. The magnitude of the GEI SS was 2.3 times larger than for rootstock genotypes (G), indicating that there were sustainable differences in genotypic (rootstock) response across environments (Ellendale selections).

Table 4.9 Mean yield (kg tree^{-1}) ranking (1-4) of the rootstock genotype (G) selections per environment (Ellendale selections) at one locality according to the AMMI model

Ellendale selections (E) Environment (Env.)	Env. mean	Score	AMMI Rootstocks (G) per Ellendale selections (E): 1	2	3	4
1033	45.59	1.91	575	608	306	42
228b	40.72	1.12	575	608	306	42
228a	54.02	1.07	575	608	42	306
1034	57.66	1.06	575	608	42	306
1032	64.44	0.60	575	42	608	306
1085	73.38	-5.76	42	575	306	608

Cultivar means, ranking order of the four rootstocks per Ellendale selection and first IPCA component scores of the rootstocks are presented in Table 4.9. Mean yields of the six environments varied from 73.38 kg tree^{-1} to 40.72 kg tree^{-1}.

4.3.3.2 Rootstock (G) genotypic and environment interaction (Group 1: Ellendale mandarin)

To determine the environment effect of production years (E) on the rootstock (G), the diversity due to citrus scion type (group effect) was removed and genotypes within a citrus scion type were thus used (Figure 4.4).

AMMI analysis for yield of four rootstocks genotypes (G) evaluated with Ellendale selections for five years (E) is presented in Table 4.10 and showed that the genotype and environment main effects were significant (P≤0.05). The model revealed that differences between the environments (production years) accounted for 93.73% of the treatment SS. Rootstock genotypes (G) and GEI also contributed significantly at 4.25 and 2.02%, respectively. The SS for the first principal component axis (IPCA) was significant at P≤0.05 and captured 72.53% of the interaction SS in 50% of the df. The other 27.47% of the variation (within 50% of the interaction df) was left in the IPCA2 and residual combined and was not significant.

Table 4.10 AMMI analysis of variance for yield of four rootstocks (G) genotypes per years (E) within the Ellendale citrus scion type, at one locality

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained		
					Total Variance	Treatment variance	Interaction components
Total	79	104213	1319				
Treatment	19	100831	5307	0.00			
Environment (years)	4	94509	23627	0.00	**90.69**	**93.73**	
Block	15	304	20	0.99	0.29		
Genotype (rootstocks)	3	4286	1429	0.00	**4.11**	**4.25**	
Interaction	**12**	**2035**	**170**	**0.01**	**1.95**	**2.02**	
IPCA 1	**6**	**1476**	**246**	**0.01**			72.53
IPCA 2	**4**	**467**	**117**	**0.17**			22.95
Residual	2	92	46	0.52			4.52
Error	45	3077	68		2.95		
					100.00		100.00

Cultivar means, ranking of the four rootstocks per production year (E) and first IPCA component scores of the rootstock genotypes are presented in Table 4.11. Mean yields of the five environments (production years) varied from 65.53 to 107.62 kg tree^{-1}.

Table 4.11 Mean yield (kg tree^{-1}) ranking (1-4) of the rootstock genotypes (G) per production year (E) in one locality analysed according to the AMMI model

Year (E)			AMMI Rootstocks (G) per production year (E)			
Environment (Env.)	Env. mean	Score	1	2	3	4
Y4	65.53	-3.64	306	608	575	42
Y2	11.28	-0.27	306	608	42	575
Y3	70.21	0.89	608	306	42	575
Y1	25.19	1.01	608	306	42	575
Y5	107.62	2.02	608	42	306	575

Figure 4.5 depicts the complex genotype x environment context of a grafted tree partitioned into G, E and GEI within the citrus scion type Ellendale. Data was obtained from the AMMI ANOVA tables 4.2, 4.4, 4.6, 4.8 and 4.10. The same reasoning and procedure were followed for mandarin, grapefruit and Valencia and the resulting data are summarised in Table 4.12.

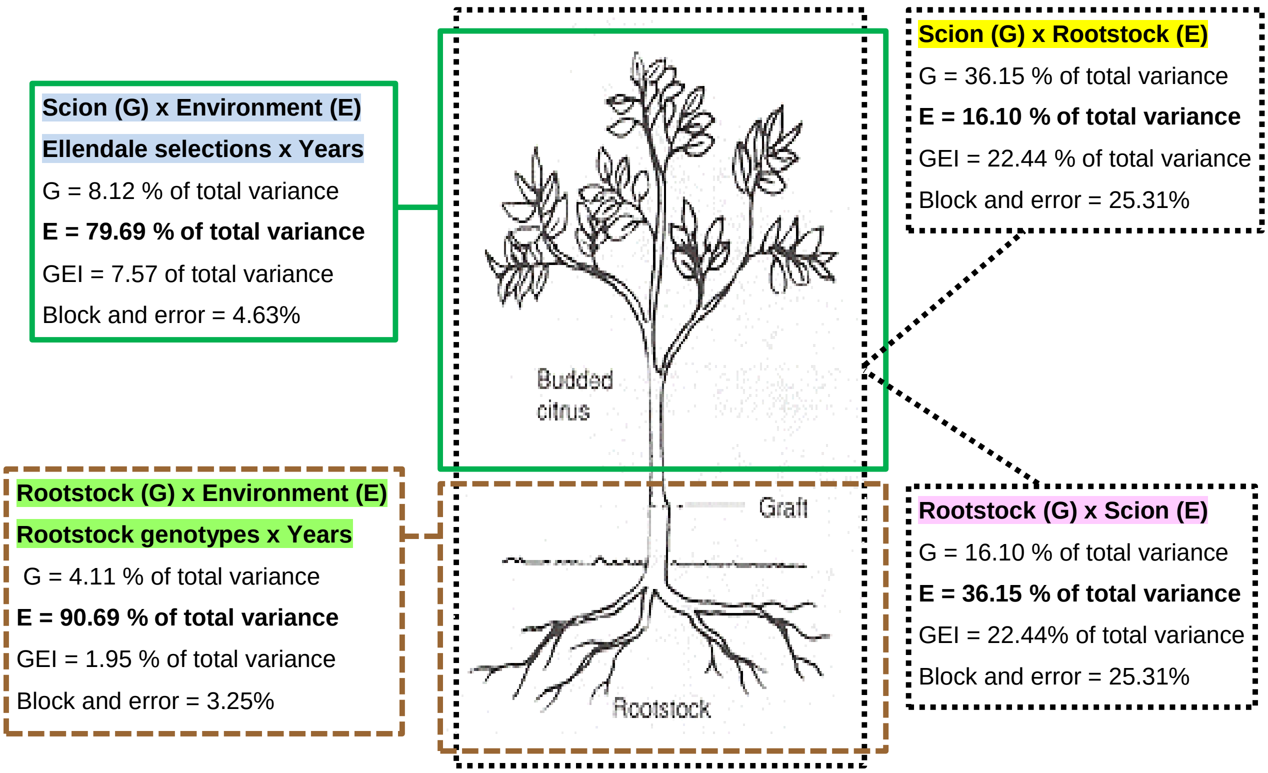

Figure 4.5 Summary of the complex genotype x environment context of a grafted tree partitioned into G, E and GEI within the citrus scion type Ellendale.

Table 4.12 Summary and comparison of variance percentages for yield accounted for by G, E and GEI within each of four citrus scion types grafted to four different rootstocks and evaluated over five years in one locality as with AMMI analysis

*Source of variance	**Stion (G) x Years (E)	Scion (G) x Rootstock (E)	Rootstock (G) x Scion (E)	Scion (G) x Years (E)	Rootstock (G) x Years (E)
	% variance explained*				
Ellendale					
Genotypes	15.19	48.39	21.55	8.51	4.25
Environments	72.14	21.55	48.39	83.55	93.73
Interactions	12.68	30.07	30.07	7.94	2.02
IPCA1	50.33	92.17	92.17	73.73	72.53
IPCA2	27.99	NS	NS	21.67	NS
Mandarin					
Genotypes	21.08	41.84	51.91	11.90	19.75
Environments	39.49	51.91	41.84	53.28	71.29
Interactions	39.43	NS	NS	34.83	8.96
IPCA1	46.00	NS	NS	58.87	80.19
IPCA2	33.11	NS	NS	27.86	15.03
Grapefruit					
Genotypes	31.13	27.50	53.21	13.83	22.97
Environments	47.15	53.21	27.50	76.20	65.38
Interactions	21.73	19.29	NS	9.96	11.66
IPCA1	58.32	53.50	NS	64.07	76.17
IPCA2	20.11	NS	NS	27.37	19.31
Valencia					
Genotypes	37.83	44.53	39.52	27.45	23.22
Environments	40.96	39.52	44.53	66.74	63.61
Interactions	21.22	15.95	15.95	5.82	13.17
IPCA1	68.04	57.24	57.24	67.11	85.59
IPCA2	19.83	NS	NS	NS	14.18

* Genotype, Environment and Interactions add to 100% but the IPCA percentages per column will not add to 100% as the residual were not included in this table.
** High residual percentages were evident in all citrus scion types for this option.

Ellendale: Stion (G) x year (E) indicated a very low G and GEI (15.19 and 12.68%) contribution to the percentage variation accounted for (Table 4.12). The AMMI ANOVA of the stion combinations tested over five years showed that genotypes and environment main effects were significant (P≤0.05) and GEI was significant (P≤0.05); suggesting that GEI influenced the performance of the stions with regard to yield in all years. The large percentage variation attributable to the environment (years) suggested that the environments (years) were diverse, with large differences among environmental means. Table 4.13 was extracted from the AMMI analysis, following the principle of Fox *et al*. (1990) that a genotype usually found in the top third of entries across environments can be considered relatively well adapted for the trait investigated. However, determining rootstock and/or scion performance relative to the environment was difficult.

Table 4.13 Mean yield (kg tree^{-1}) ranking (1-4) of stions (G) per year (E) within the Ellendale citrus scion type at one locality analysed according to the AMMI model

Year			AMMI stion selections			
Env.	Env. mean	Score	1	2	3	4
Yr. 5	107.62	-8.084	1085 on 575	1085 on 42	1085 on 306	1032 on 575
Yr. 1	25.19	-1.207	1085 on 42	1085 on 575	1032 on 575	1085 on 306
Yr. 4	65.53	-0.171	1085 on 42	1085 on 42	1032 on 306	228a on 575
Yr. 3	70.21	4.302	1032 on 575	1085 on 42	1032 on 306	228a on 575
Yr. 2	11.28	5.16	1085 on 42	1032 on 42	1034 on 575	228a on 42

Partitioning of the scion and the rootstock resulted in 30.07% of the variation being attributed to interaction amongst the scions and rootstocks. Table 4.12 indicated that there was substantial interaction amongst the Ellendale selections and rootstocks. However, there was less interaction between the rootstocks and years (E) than between the scions and years (E).

Table 4.14 shows a comparison of the rankings of the top three genotypes (G) for mean yield (kg tree^{-1}), per environment (E) for the partitioning of the rootstock and scion effects for Ellendale, mandarin, grapefruit, and Valencia.

From Table 4.14 it was evident that 575 with a score close to zero was a stable high yielding rootstock. It also manifested in the top third of rootstocks over the five production years (E). Ellendale selections (G) 1085 and 1032 were found in the top third of selections per rootstock and years (except for year 2) as environments. No crossover was detected with regard to scions on 42, 575 and 306. Scions as environments for rootstocks were more diverse with 1033 and 228b, 1034 and 228a grouping together while selections 1085 and 1032 were on their own. With regard to the interaction of scion with year, three mega-environments were identified. Year one, four and five were conducive to the same genotypes while year two and three differed from this group as well as from each other.

Table 4.14 Comparison of the rankings of the top three genotypes (G) for mean yield (kg tree^{-1}), per environment (E) for the partitioning of the rootstock and scion effects per citrus scion types Ellendale, mandarin, grapefruit, and Valencia

Rootstock (G) x Scion (E)

ENV	EM	Score	AMMI rootstock 1	2	3
Ellendale					
1085	73.4	-5.76	42	575	306
1032	64.4	0.60	575	42	608
1034	57.7	1.06	575	608	42
228a	54.0	1.07	575	608	42
228b	40.7	1.12	575	608	306
1033	45.6	1.91	575	608	306
Mandarin					
1082	51.3	-1.80	42	575	306
1211	26.3	-1.04	42	306	575
918	42.1	0.10	42	575	608
803a	42.1	2.73	42	575	306
Grapefruit					
1057a	157.7	-2.51	575	608	42
1053	129.5	-2.14	575	608	42
1069	136.4	-2.05	575	608	42
1179	122.1	-1.67	575	608	42
1176	137.9	-1.41	575	608	306
231	141.1	1.17	575	608	42
1058	141.9	1.31	575	42	608
1057b	112.0	1.80	575	306	608
1183	105.1	5.50	575	42	306
Valencia					
1043b	45.9	-2.79	575	42	608
1060	91.3	-2.71	575	42	608
1043a	89.3	-1.08	575	608	42
1056	98.8	0.06	575	608	306
1063	98.2	0.26	575	608	306
1044	57.1	0.28	575	608	306
1055	90.0	0.46	575	306	608
1052	87.2	5.52	575	306	42

Rootstock (G) x Year (E)

ENV	EM	Score	AMMI selections 1	2	3
Ellendale					
Yr. 4	65.5	-3.64	42	575	608
Yr. 2	11.3	-0.27	575	42	608
Yr. 3	70.2	0.89	575	42	306
Yr. 1	25.2	1.01	575	42	306
Yr. 5	107.6	2.02	575	306	42
Mandarin					
Yr. 3	59.3	-3.48	42	306	575
Yr. 5	67.1	-2.04	42	306	575
Yr. 4	27.7	1.38	42	575	306
Yr. 1	28.7	1.70	42	575	306
Yr. 2	19.3	2.45	42	575	608
Grapefruit					
Yr. 2	168.4	-5.93	575	42	608
Yr. 4	101.5	-0.46	575	608	42
Yr. 3	157.9	0.58	575	608	42
Yr. 1	76.3	1.06	575	608	42
Yr. 5	153.6	4.75	575	608	306
Valencia					
Yr .5	117.0	-6.27	575	306	608
Yr. 4	90.7	-0.21	575	608	306
Yr. 3	105.7	1.54	575	42	306
Yr.1	53.9	2.46	575	42	608
Yr.2	43.9	2.48	575	42	608

Scion (G) x Rootstock (E)

ENV	EM	Score	AMMI selections 1	2	3
Ellendale					
42	58.5	-4.49	1085	1032	1034
575	66.8	-0.24	1085	1032	1034
306	48.8	0.22	1085	1032	1034
608	49.8	4.51	1032	1034	228a
Mandarin					
575	40.5	-2.23	803a	1082	918
306	35.9	-1.01	1082	803a	918
608	29.1	1.13	1082	918	803a
42	56.3	2.12	1082	918	803a
Grapefruit					
608	136.7	-6.31	1057a	1069	1176
575	163.7	0.62	1057a	231	1176
306	107.0	2.80	231	1176	1057a
42	118.8	2.90	1057a	1058	231
Valencia					
608	74.5	-3.74	1056	1063	1043a
42	69.6	-3.06	1060	1063	1056
306	72.8	2.87	1052	1063	1060
575	112.0	3.93	1056	1052	1063

Scion (G) x Year (E)

ENV	EM	Score	AMMI selections 1	2	3
Ellendale					
Yr.5	107.6	-5.48	1085	1032	1034
Yr.1	25.2	-0.65	1085	1032	1034
Yr.4	65.5	-0.08	1085	1034	1032
Yr.3	70.2	2.88	1032	228a	1034
Yr.2	11.3	3.33	228a	1034	1033
Mandarin					
Yr.4	27.7	-6.05	1082	1211	803a
Yr.5	67.1	-0.48	1082	803a	918
Yr.3	59.3	1.30	1082	803a	918
Yr.2	19.3	2.05	918	1211	803a
Yr.1	28.7	3.18	918	803a	1082
Grapefruit					
Yr.2	168.4	4.86	231	1057a	1058
Yr.3	157.9	0.87	1057a	1069	1176
Yr.5	153.6	-3.41	1057a	231	1058
Yr.4	101.4	-5.12	1057a	1176	1058
Yr.1	76.3	2.80	1069	1057a	1176
Valencia					
Yr.5	117.0	-4.28	1043a	1063	1056
Yr.4	90.7	-1.37	1056	1063	1060
Yr.2	43.9	-0.25	1056	1063	1060
Yr.1	53.9	1.21	1063	1056	1055
Yr.3	105.7	4.70	1055	1063	1060

ENV = Environment, EM = Environment mean score = AMMI IPCA1 score for environment and AMMI selections = 1-3 of the first four AMMI selections per environment. Grey blocks represent non-significant GEI as per AMMI analysis.

Mandarins: Data showed that variation for yield within the mandarin citrus scion type was nearly equally attributable to the scion and the rootstock at 51.91 and 41.84% of the treatment SS respectively within a stion combination, within the group (Table 4.12). There were no significant interactions amongst the scions and the rootstocks. Environments (years) accounted for 53.28 and 71.29% of the treatment variation within the scion x year and rootstocks x year combinations respectively. Environment (years) and GEI (39.49 and 39.43%) accounted in equal parts for the variation with regard to stion x year (Table 4.12). GEI for scion x year was very high at 34.83%. Rootstock genotypes were less interactive with the environment at 8.96%

Grapefruit: The most interesting results pertain to the grapefruit group (Table 4.12). The model revealed for the Scion (G) x Rootstock (E) that differences between the environments (rootstocks) accounted for 53.21% of the treatment SS. Genotypes (scions) and GEI also contributed significantly to variation at 27.50 and 19.29% respectively. The SS for the first principal component axis (IPCA1) was significant at $P<0.05$ and captured 53.50% of the interaction SS in 41.16% of the df. IPCA2 and residual were not significant. When rootstocks were deemed the genotype and the scions the environment, the magnitude of the genotype SS was 2.76 times larger than that for GEI. The AMMI analysis showed GEI in this case not to be significant.

Valencia: AMMI ANOVA percentage variance for yield accounted for by G, E and GEI of selections within Valencia group grafted to four different rootstocks and evaluated over five years in one locality showed that the scion (Valencia selections) and the rootstock as main effects contributed almost equally to the total SS. Interaction between the scions and the rootstocks accounted for 15.95 of the treatment SS (Table 4.12). The result of the AMMI analysis showed that 66.74 and 63.61% of the treatment SS for the scion and rootstocks respectively were attributable to the effect of production years (environmental effects). However, GEI for scion by environment interaction in Valencia was smaller (5.82%) than the rootstock by environment interaction (13.17%). The IPCA1 was significant ($P\leq0.05$) in all instances but the IPCA2 was only significant for rootstock by environment (years) where the IPCA1 and IPCA2 combined explained 99.76% (85.59 +14.18%) of the variation (within 83.33% of the interaction df) of the GEI.

Table 4.12 and Table 4.14 were highly informative regarding the rootstocks and scions tested. GEI was found through AMMI analysis to be significant in all instances except rootstock (G) by scion (E) for grapefruit and mandarin and for scion (G) by rootstock for mandarin.

4.4 DISCUSSION

Noise and interaction amongst the genotypes and environments are two fundamental problems to be solved in any trial aimed at selecting superior genotypes in a breeding programme or at recommending new cultivars to farmers (Gauch and Zobel, 1996). AMMI was found to address both of these problems. Noise can be countered by sound trial layouts and management. With regard to GEI two approaches exists, one aimed at genotypes by seeking high yielding, widely adapted genotypes and the other at environments by identifying homogenous macro environments and then to breed and recommend cultivars specifically for these environments.

4.4.1 Defining rootstock (G) with regard to citrus scion type effect on rootstock

For the first question pertaining to whether there is any GEI with regard to rootstock grafted to very different citrus scion types it was found that GEI was significant and that rootstock evaluation should be specific to each mega-environment (citrus scion type).

According to Farshadfar *et al*. (2011), a genotype/environment that has a large positive IPCA score with some environments/genotypes must have negative interaction with some other environments/genotypes. Grapefruit was the highest yielding citrus scion type with a large negative value (Table 4.3), thus being a very discriminating environment with regard to rootstocks. Valencia, with the second highest mean yield had a high negative IPCA1 score and a high positive IPCA2 score (Table 4.3). These scores presented a disproportionate genotype to environment response (Yan and Hunt, 2001; Mohammadi *et al*., 2007), which was the major source of variation for any crossover (qualitative) interaction.

In contrast, a proportionate genotype response or a non-crossover (quantitative) GEI will be represented by scores with the same sign or near zero (Mohammadi and Amri, 2008; Farshadfar *et al*., 2011). Genotypes and environments with PCA 1 scores of zero or near zero have small interaction (Crossa, 1990). Thus regarding rootstocks reaction per citrus scion type none of the environments (citrus scion types) proved to be a stable high yielding species for none of the scores were close to zero (Table 4.3).

However, relating to mega-environments, ideal test environments should have large IPCA1 scores (better at discriminating the genotypes) and near zero IPCA2 scores (more representative of an average environment) (Yan, 1999; Yan *et al*., 2000). In this regard, mandarin had a large positive value and an IPCA2 score of 0.95, which was the closest IPCA2 score to zero of all the groups (Table 4.3). The GEI for Scion x Rootstock also proved to be non-significant (Tabale 4.12), deeming the mandarins a discriminating and stable mega-environment for testing new rootstocks.

Significant GEI and crossover (qualitative) interaction for the other citrus scion types concluded that the citrus scion types were indeed mega-environments except for midseason oranges, which had almost an identical score within the same range to the Ellendale citrus scion type, which indicates that rootstock evaluation should be specific to each mega-environment (citrus scion type). The high yielding citrus scion types namely grapefruit and Valencia were more associated with rootstocks 575 and 608 while Ellendale (including midseason oranges) and mandarins were more associated with rootstocks 575 and 42.

4.4.2 Separating the stion GEI into a scion GEI and a rootstock GEI for yield per mega-environment (citrus scion type)

The objective was to separate and quantify the contribution of the scion and rootstock to the stion in a single physical environment (i.e same climate, same soil, same production practices).

When stion x year was analysed the genotype accounted for 15.19% of the variance in yield for Ellendale (Table 4.12). However, partitioning the stion in a scion and rootstock revealed that 48.39% of the variance in yield in the stion was accounted for by the scion (Ellendale selections) and the other 21.55% by the rootstock. It was also found that in the scion x year and rootstock x year, the environment actually accounted for the expected 80 to 90% of the variance (Table 4.12). As the variance accounted for within the stion was mostly due to the Ellendale selection, it is interesting to note that there was also a differential genotypic response of scions to environments (years) in Ellendale, while the rootstocks accounted for very little of the variance measured with regard to years (Table 4.12). Ellendale scions were thus both sensitive to the rootstock used as well as physical environment (years) of the scions.

Scion (G) x year (E) in Ellendale was the only instance in this investigation where the E:G:GEI adhered to the expected 70:10:20 principle as postulated by Gauch and Zobel (1996) for field trials in different agro-ecological conditions.

Rootstock scion interactions were non-significant and thus negligible in mandarins but significant in all of the other citrus scion types (Table 4.12). All the interaction (39.43%) accounted for within the mandarin stions (Table 4.12) were thus due to the year effects. Mandarin scions were also more susceptible to year effects (34.83%) than the mandarin rootstocks (8.96%).

In contrast to the no-interaction detected amongst scions and rootstocks in mandarins, a large SS for environments with regard to scion x year indicated that the years were diverse, with large differences among environmental means causing most of the variation in yield. The magnitude of the SS of the GEI was 2.9 times larger than that of the scions (G), indicating that there were sustainable differences in genotypic response of the mandarins over years. Percentage variation explained by GEI for the rootstocks (G) x year (E) were significant but not as pronounced.

As was expected the scion contribution was found to be more prominent than the rootstock contribution to yield in all citrus scion types except in grapefruits where the rootstocks were responsible for 53.21% of the yield effect as opposed to the 27.50% contribution of the scion. GEI regarding scion (G) x rootstock (E) for grapefruit was significant but not for rootstock (G) x scion (E). This implies that the rootstocks in some or another way influenced the scions whereas the scions had no significant influence on the rootstock. Year effect with regard to rootstocks (G) grafted to grapefruits were less pronounced with a magnitude of 2.8 (65.38÷22.97) times larger than the genotype effect in comparison to the year effect (which was 5.5 times larger) with regard to the scion (G) effect.

Where the percentage variation accounted for by the scions was almost twice that of the rootstocks with a fairly sizable interaction amongst rootstocks and scions in Ellendale, the contribution of scion and rootstock for Valencia was almost equal but the percentage variation accounted for by interactions amongst rootstocks and scions was half of that of the Ellendale group.

When genotypic main effects were the only consideration (Chapter 3) the industry standard cultivar for Valencia namely 1043, and new selection 1044 were constantly ranking lower than the other cultivars with significantly lower yield, with the rest of the selections not differing significantly from each other. Although 'McClean'-1056 always ended up being the top producer, it did not differ statistically from the other cultivars tested. However, following the AMMI procedure considering GEI, that included rootstock scion interactions, it was found that Valencia selections 1056 and 1063 were the most suitable to all rootstocks as it had IPCA scores close to zero indicating them to be more stable genotypes across environments (rootstocks) such as 575 (Table 4.14). Selection 1052 proved to be a highly discriminating environment for rootstock testing but with its IPCA value also being high it did not prove to be a representative environment.

As could be seen from the data a lot of deduction is needed to interpret the AMMI results confirming Mohammadi's (2012) statement that the AMMI model does not make provision for a quantitative stability measure and that such a measure is essential in order to quantify and rank genotypes according to their yield stability.

4.5 CONCLUSION

The purpose of test-environment evaluation is to identify test environments that effectively identify superior genotypes for a mega-environment. An "ideal" test environment should be both discriminating of the genotypes and representative of the mega-environment (Yan *et al*., 2007). The existence of GEI complicates the identification of superior genotypes for a range of environments and calls for the evaluation of genotypes in many environments to determine their true genetic potential.

Regarding the first question pertaining to whether there is any GEI with regard to rootstock grafted to very different citrus scion types, it was found that GEI was significant and that rootstock evaluation should be specific to each mega-environment (citrus scion type).

It was also determined that stion GEI can successfully be separated into a scion GEI and a rootstock GEI for yield per mega-environment (citrus scion type). With regard to mandarins, it was determined that there is no interaction with regard to scion and rootstock thus mandarin evaluation can take place on any of the rootstocks in this trial. However, with regard to interaction of the rootstocks and scions with environment (year) and the impact thereof on the stion, it was substantial.

Cultivar or selection ranking can differ greatly across environments due to interactions or differential genotypic responses to environments. In long term field trials the aim of GEI analysis would be to determine the stability of the genotypes especially when there is a reasonable (GEI). As the AMMI model does not make provision for a quantitative stability measure, a lot of deduction is needed to interpret the AMMI results. A quantitative stability measure is essential in order to quantify and rank genotypes according to their yield stability.

4.6 REFERENCES

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Chapter 5

RELEVANCE OF AMMI FOR INVESTIGATION GEI PERTAINING TO QUALITY AMONGST CITRUS SCIONS, ROOTSTOCKS AND ENVIRONMENTS

Abstract

At the first level, fruit quality is simply the sum of those attributes that create and enhance consumer appeal. Attributes of interest to the consumer that create and enhance appeal are general visual appearance (such as rind colour, size and blemishes), texture, firmness, sensory and lately also include nutritional and food safety attributes. It is thus evident that fruit growers cannot depend primarily on yield to determine their net income but also on fruit quality as dictated by the consumer. Ability of fruit growers to achieve profitable levels of productivity and fruit quality is, in turn, largely a matter of the environment, which includes climate as well as the orchard system selected and the successful management thereof. The problem with grafted trees is that rootstock performance is an indirect assessment with its performance reference being that of the scion grafted on it. A single measurement can thus be attributed to the rootstock's reaction to the environment as well as the rootstock's interaction with the scion and vice versa, as has been illustrated in Figure 4.1 in Chapter 4. By determining these interactions, it might be possible to determine and manipulate the actual contribution of the rootstock's genotype with regard to a specific attribute. This will have a significant influence on the size and extent of rootstock and scion trials in pursuit of broad and /or specific adapted cultivars. Knowledge pertaining to rootstock scion interactions can also help to reduce trial numbers and size when testing rootstocks by identifying citrus scion types and planting locations as test environments for future rootstock evaluations. Such as group/location should be representative of the target population (citrus scions/citrus locations) yet highly discriminative to distinguish the best rootstocks with regard to quality attributes. With regard to being both discriminative and stable for all the traits in this trial, no single mega environment for rootstock selection could be found. The five citrus scion types were thus indeed mega environments with regard to quality attributes and rootstock evaluation should be specific to each mega environment (citrus scion type). Mega environments were thus trait specific. For example, Valencia was the most stable with regard to TSS:Acid ratio while grapefruit represented the ideal test environment for peel thickness. By assessing the GE data for the traits within a single mega-environment, the contribution of the scion and the rootstock to the phenotype of the stion was successfully separated and quantified. With regard to peel thickness in grapefruit, the rootstock contributed more (54.96%) towards the phenotype of peel thickness than the scion (38.23%) with no significant interaction between the rootstocks and scions. The existence of GEI amongst rootstocks and scions can either complicate or facilitate the identification of superior genotypes for a range of environments. By

knowing the nature and magnitude of the GEI the potential causes can and should be explored for the purpose of mitigation or exploitation but this requires covariates to be included in the study.

5.1 INTRODUCTION

The biological basis of fruit quality is a complex interchange of molecular, physiological, and biochemical processes including carbohydrate, protein and lipid metabolism, the synthesis and breakdown of pigments and structural components, and the formation of volatiles, that all contribute to the development, maturation or ripening, and senescence of the fruit (Passam *et al*., 2011).

However, according to Castle (1995) it is convenient to consider fruit quality at three levels: simple, complex, and specific. At the first level, fruit quality is simply the sum of those attributes that create and enhance consumer appeal.

Attributes of interest to the consumer that create and enhance appeal are general visual appearance (rind colour, size, blemishes etc.), texture, firmness, sensory and lately also include nutritional and food safety attributes. In order to reduce variation, instrumental measurements are preferred over sensory evaluations for many research and commercial applications. Instruments are more precise, and can provide a common language among researchers, industry and consumers (Abbott, 1999). Human perception of sensory attributes is guided by many factors and therefor is difficult to analyse objectively. Acceptable taste of citrus fruits, for instance, is mainly the result of the proper blending of sugars and acids (Ladaniya, 2008) determined by the relationship between the total soluble solid content and the titratable acidity (Marcilla *et al*., 2006). This is probably an over simplified approach as fruit flavour is composed of complex combinations of soluble and volatile compounds. In citrus several low-abundance sesquiterpenes, such as valencene, nootkatone, alpha-sinensal, and beta-sinensal, stand out as important flavour and aroma compounds (Sharon-Asa *et al*., 2003). Taste can also subconsciously be influenced by the appearance and smell of the fruit.

Therefore, it happens that empirical methods developed to measure some particular quality attribute actually measure maturity (Abbott, 1999). Governments in most citrus producing regions have established maturity laws requiring that citrus fruits meet certain quality standards before they can be shipped or sold in order to prevent the shipment of immature and unpalatable fruit. Laws differ slightly from country to country, but they recognize the same factors as criteria for quality which include size, shape, rind colour, peel thickness, fruit firmness, juice percentage, total soluble solids (TSS), titratable acids (TA), TSS:TA ratio, flavour, ease of peeling and seed content (Wardowski *et al*., 1995; Lacey *et al*., 2009; OECD, 2010).

It is thus evident that fruit growers cannot depend primarily on yield to determine their net income but also have to take fruit quality as dictated by the consumer, into account. Ability of fruit growers to achieve profitable levels of productivity and fruit quality is, in turn, largely a matter of the environment, which includes climate as well as the orchard system selected and the successful management thereof. Barritt (1987) defined an orchard system as: "the integration of all the horticultural factors involved in establishing and maintaining a planting of fruit trees" and include the rootstock used as an integral part of the orchard system.

Fruit quality is foremost an inherent scion cultivar trait that can be modified but not radically changed without genetic manipulation (Castle, 1995). It would therefore not be reasonable to expect those factors that modify fruit quality to have effects of large magnitude as they are of secondary importance and aimed at fine-tuning.

Development of cultivars with high and stable genetic potential for quality is one of the main goals of a fruit-breeding programme. However, this is also one of the main challenges of a fruit-breeding programme as fruit quality is complex due to the different gene actions and interactions with the environment, that is, different reactions of diverse genotypes to the same environment or different reactions of the same genotype to dissimilar or changing environmental conditions. Expression of fruit quality, precocity and maturity effects are achieved through complex interrelationship between the roots and canopy of the plant or in other words the root-shoot communication (Aloni *et al*., 2010).

In many different species, fruit quality is one of the most economically relevant traits that is influenced tremendously by rootstocks (Koepke and Dhingra 2013). Zekri (2011) states that within fairly broad parameters of adequate soil and reasonably good cultural and crop protection practices, climate is the most important component of the climate-soil-culture complex, causing differences in fruit quality among commercial citrus production areas.
Rind colour, for instance, is a major problem in the tropics as the attractive rind colour is determined by cool temperatures while the autumn decline in air and soil temperature

marks the onset of colour changes in subtropical regions. High temperatures and high humidity can result in rapidly senescing fruit that is highly susceptible to blemishes and have a poor storage capacity. Internal quality is also affected by climate. Fruit developing in a hot, tropical climate tends to have high total soluble solids (TSS) content, which is an advantage for the processing industry (Ladaniya, 2008). Fruit developing under warmer climates reach marketable sugar/acid ratios sooner than fruit developing in cooler localities but it can also often result in fruit that are low in acid, resulting in poor eating quality. High night temperatures such as that of the tropical regions result in low acid fruit, while fruit produced where night temperatures are low, is highly acid. Thus somewhat cooler, subtropical conditions are preferable for the production of oranges and mandarins for the fresh fruit market (Goldschmidt, 1997).

It has long been established that fruit grown in humid areas has a thinner peel than those grown in desert areas. In general, fruit produced in hot, desert climates versus those in hot, wet climates have thicker rinds and are less juicy. Low average minimum winter temperatures also caused Marsh grapefruit to have thick peels during the following summer (Cohen *et al*., 1972).

Furthermore, tree size, fruit quality, precocity, fruit production and maturity are achieved through complex interrelationship between the roots and canopy of the plant (Ahmed *et al*., 2006). Jahromi *et al*. (2012) states that more than 20 horticultural characteristics are affected by the rootstock, including leaf nutrient status, vigour and size, depth of rooting, low temperature tolerance, adaptation to adverse soil conditions, disease resistance and fruit quality.

In fruit farming, such as with trees and vines, choice of cultivar is an expensive and long-term investment. New fruit cultivars are pursued by producers in an effort to enhance their export market share but the essential question to be answered before a new cultivar is adopted is the long-term suitability of the cultivar to the farmers' environment. The influence of rootstocks as well as climate on yield and fruit quality in citrus is thus the reason for genetic material (newly bred cultivars) to be assessed on different rootstocks over a number of years for a certain area and even sometimes in more than one locality (multi-environment trials). The genotype's phenotypic expression of a trait in a specific environment consists of genotypic main effects (G), environment effects (E) and the interaction of the two. According to Yan and Tinker (2005) the only way that complex GEI can be exploited is by dividing target environments into meaningful mega-environments.

The problem with grafted trees is that rootstock performance is an indirect assessment with its performance reference being that of the scion grafted on it. A single measurement can thus be attributed to the rootstock's reaction to the environment as well as the rootstock's interaction with the scion and *vice versa,* as has been illustrated in Figure 4.1 in Chapter 4. By determining these interactions, it might be possible to determine and manipulate the actual contribution of the rootstock's genotype with regard to a specific attribute. This will have a significant influence on the size and extent of rootstock and scion trials in pursuit of broad and /or specific adapted cultivars.

The original definition for a mega-environment refers to a portion of a crop species' growing region with a homogeneous environment that will cause genotypes to perform similarly (Gauch and Zobel, 1997). As a stion's performance is measured in relation to a specific environment, a rootstock's performance is measured relevant to a scion and therefore mega-environments for rootstocks in a single physical environment would pertain to scions.

The objective of this study was thus to use the AMMI model to determine:

a) whether the five citrus scion types pre-identified based on the species and time of ripening within a species namely *C. sinensis* (two types: midseason and Valencia), *C. paradisi* and *C. reticulata* (two types: early and late) are indeed mega-environments in the target group of total citrus with regard to some quality aspects.
b) whether it is possible to partition the reaction of the stion into scion and rootstock reactions with regard to quality aspects. For this purpose peel thickness was chosen, due to the fact that the *Citrus spp.* exhibits a pronounced range in thickness from the extremely thick rind of some of the pummelos and the citrons to the very thin peel of the Indian acid lime. The range within each fruit group is also variable, giving rise to both thin-skinned and thick-rind varieties.

5.2 MATERIALS AND METHODS

5.2.1 Materials

5.2.1.1 Localities, scion and rootstock material

Data were taken from the same trial site and years as discussed in Chapter 4. The propagation methods, orchard practices, trial layout, scions and rootstock genotypes were thus the same.

5.2.2 Methods

5.2.2.1 Evaluation of traits

A budded citrus tree generally starts bearing within two years after planting. Evaluation is done annually as soon as the fruit reaches harvest maturity. This study was thus carried out on five citrus scion types at one locality. Trees were evaluated for five consecutive years commencing from the fourth year after plant. A representative fruit sample of 10 fruit was picked from each of the 16 trees per scion genotype. A sample of 10 fruit consisted of two inside fruit, and three outside fruit (high, shoulder height and low) taken on both the eastern and western side of the tree. Fruit weight, juice weight, peel (rind) thickness, total soluble solids, and titratable acidity were measured at the time of maturity of the control cultivar for each citrus scion type (Hardy and Sanderson, 2010).

Fruit was sectioned equatorially in order for peel thickness to be measured with an electronic hand calliper and the juice was extracted by hand with a Pineware CS2 citrus juicer. Total soluble solids were determined with a temperature-compensating digital refractometer (Palette PR-101) and titratable acidity by titration of a 10 ml aliquot of juice using 0.156N NaOH to an endpoint with phenolphthalein as an indicator (Wardowski *et al*., 1995).

5.2.2.2 Statistical analysis

GEI for fruit quality was analysed according to a classical multiplicative model or AMMI (Gauch, 1992) with two multiplicative terms as discussed in Chapter 4. AMMI analysis was constructed using GenStat 15th edition (Payne *et al*., 2012). Shapiro-Wilk test was performed to test for normality of the residuals (Shapiro and Wilk, 1965).

5.2.2.3 Logic and procedure to partitioning the stion GEI into scion GEI and rootstock GEI for quality

A procedure for successful partitioning of the stion GEI into a scion GEI and rootstock GEI is illustrated in Figure 5.1.

If there is no GEI for fruit quality it would not be necessary to conduct trials in multiple environments and to analyse G by E data (Yan and Tinker, 2006). The first step would thus be the quantification of the magnitude and nature of GEI contained in the data. If no significant GE exists in the data, any single environment would suffice for reliable evaluation of the genotypes. However, the more complexity there is in the genetic system underlying the trait, the more it is prone to GEI with the consequence that different winners can be identified in different environments. This is called crossover interaction.

In the case of non-crossover interaction, superior genotypes can be identified in any of the environments with the difference that an ideal test environment exists in which the best genotypes can be more effectively identified. Repeatability across years should be verified. If not repeatable with no recognizable pattern of GE, the target environment is a single mega environment with unpredictable GE and genotypes should be selected on mean and stability. Repeatable interactions imply that the target environments should be divided into different mega-environments and genotype evaluation should be conducted separately for each mega-environment.

According to Yan and Tinker (2006), the objectives of data analysis within a single mega-environment are twofold: test environment evaluation to identify test environments that are both informative (discriminating) and representative as well as genotype evaluation to identify genotypes with both high performance and high stability.

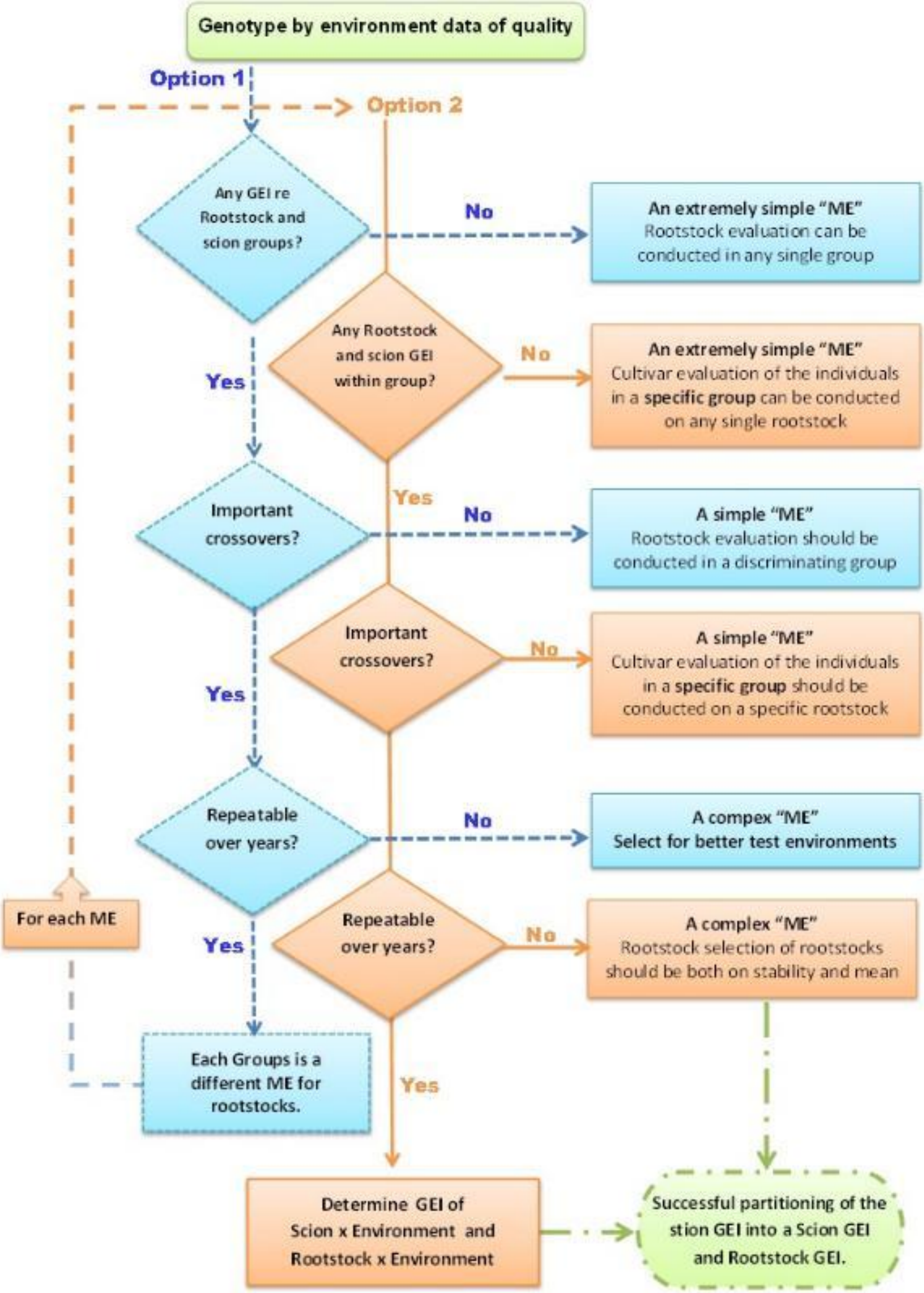

Figure 5.1 **A procedure for successful partitioning of the stion GEI into a scion GEI and rootstock GEI (ME= mega environment)**

In order to determine the influence of the scion genotype on the rootstock genotype the rootstocks were grafted with five very different citrus scion types namely *C. sinensis* (two types: midseason and Valencia), *C. paradisi* and *C. reticulata* (two types: early and late). The first option investigated was whether there is any GEI between the scions and the rootstocks with regard to quality aspects. In this case, citrus scion types were considered environments (Figure 5.2).

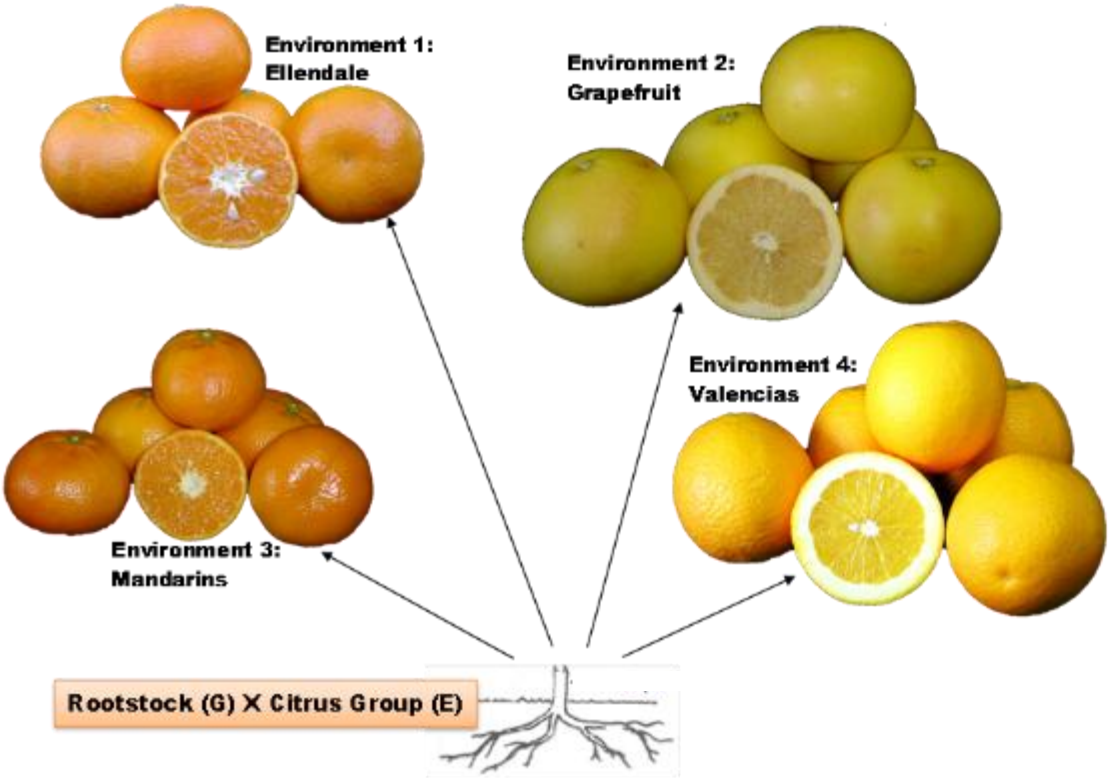

Figure 5.2 An illustration of partitioning the stion GEI by defining the rootstock (G) in different environments (citrus scion types) with regard to quality

Significant GEI between rootstocks and scions will lead to further investigations via Option 2. In Option 1 citrus scion types were the environment but in Option 2 the scions within a citrus scion type were the environments thus partitioning the stion GEI by defining the rootstock (G) in different environments (scions and years) thus scion effect on rootstock and year effects on rootstock as illustrated in Figure 4.4 (Chapter 4).

Figure 4.3 (Chapter 4) illustrates the partitioning of the stion's GEI by defining the scion (G) in different environments (rootstocks) thus rootstock effect on scion and year effects on scion for fruit quality within a citrus scion type (e.g. Ellendale). The scenario in Figure 4.3 elucidates the role of the rootstock within the stion and its' reaction in relation to the different scions and the environment, thus the performance of the stion as influenced by the rootstock and the environment will be clarified.

If the data identify a meaningful GEI it will indicate that there is a differential genotypic environment interaction. In a standard ANOVA procedure a series of (G) genotypes in (E) environments would result in an interaction SS with (G-1)(E-1) df which could be difficult to interpret (Smith, 1992) as was illustrated in Chapter 3.

With the AMMI model, each environment is characterised with an environment 'score' for each component. Correspondingly, genotype performance is characterised for each component with a genotype score. Experimental results can now be explained via the weighted sum of products of such scores and cultivar response in differing environments can even be predicted via the model.

5.3 RESULTS

5.3.1 Defining rootstock (G) with regard to citrus scion type effect on rootstock

When field trials are carried out in different agro-ecological conditions, usually 80% of variation is caused by environment, while genotype and GEI usually cause 10% of variation each (Yan, 2001). AMMI ANOVA for fruit quality traits (fruit mass, peel thickness, juice percentage, TSS, TA and TSS:TA ratio) of four rootstocks genotypes evaluated with five different citrus scion types is presented in Table 5.1 and showed significant effects for genotype, environment and GEI with regard to fruit quality traits for all five citrus scion types.

With regard to juice percentage, as an example, the results showed that 89.17% of the total SS was attributed to environmental effects, while only 2.10 and 6.55% variation was attributed to genotype and GEI effects respectively (Table 5.1). Results from the AMMI analysis for juice percentage showed that the SS for the first and second principal component axes (IPCA1 and 2) together captured 98.12% of the interaction SS in 83% of the df and was significant at $P<0.05$.

Table 5.1 AMMI analysis of variance of four rootstock genotypes (G) evaluated with five different citrus scion types (E) for various quality attributes

FRUIT MASS

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained: Total Varian	% Variance explained: Interaction component
Total	79	20318	2572			
Treatment	19	19753	1039	0.00		
Environment	4	19135	4784	0.00	**94.18**	
Block	15	2008	134	0.10	0.99	
Genotype	3	276	92	0.34	**0.14**	
Interaction	**12**	**5904**	**492**	**0.00**	**2.91**	
IPCA1	**6**	**4483**	**747**	**0.00**		**75.93**
IPCA2	**4**	**1352**	**338**	**0.01**		22.90
Residual	2	69	34	0.66		1.17
Error	45	3636	81		1.79	

FRUIT TSS

Source of variance	Df	SS	MS	Probability (P≤0.05)	% Variance explained: Total Variance	% Variance explained: Interaction component
Total	79	153.87	1.95			
Treatment	19	152.38	8.02	0.00		
Environment	4	144.21	36.05	0.00	**93.72**	
Block	15	0.48	0.03	0.17	0.31	
Genotype	3	4.52	1.51	0.00	**2.94**	
Interaction	**12**	**3.66**	**0.31**	**0.00**	**2.38**	
IPCA1	**6**	**2.21**	**0.37**	**0.00**		**60.38**
IPCA2	**4**	**1.36**	**0.34**	**0.00**		37.16
Residual	2	0.09	0.05	0.14		2.46
Error	45	1.00	0.02		0.65	

FRUIT PEEL THICKNESS

Source of variance	df	SS	MS	Probability (P≤0.05)	Total Varian	Interaction component
Total	7	176.74	2.24			
Treatment	1	175.86	9.26	0.00		
Environment	4	172.04	43.01	0.00	**97.34**	
Block	1	0.16	0.01	0.80	0.09	
Genotype	3	2.14	0.71	0.00	**1.21**	
Interaction	**1**	**1.67**	**0.14**	**0.00**	**0.94**	
IPCA1	**6**	**1.63**	**0.27**	**0.00**		**97.60**
IPCA2	**4**	**0.04**	**0.01**	**0.68**		2.40
Residual	2	0.00	0.00	0.88		0.00
Error	4	0.72	0.016		0.41	

FRUIT TITRATABLE ACID (TA)

Source of variance	Df	SS	MS	Probability (P≤0.05)	Total Variance	Interaction component
Total	79	3.22	0.04			
Treatment	19	3.15	0.17	0.00		
Environment	4	3.02	0.75	0.00	**93.79**	
Block	15	0.01	0.00	0.71	0.44	
Genotype	3	0.04	0.01	0.00	**1.24**	
Interaction	**12**	**0.09**	**0.01**	**0.00**	**2.81**	
IPCA1	**6**	**0.08**	**0.01**	**0.00**		**87.72**
IPCA2	**4**	**0.01**	**0.00**	**0.21**		8.41
Residual	2	0.00	0.00	0.26		3.76
Error	45	0.06	0.00		1.72	

FRUIT JUICE PERCENTAGE

Source of variance	df	SS	MS	Probability (P≤0.05)	Total Varian	Interaction component
Total	7	1592.9	20.2			
Treatment	1	1558.3	82.0	0.00		
Environment	4	1420.4	355.1	0.00	**89.17**	
Block	1	12.8	0.9	0.08	0.80	
Genotype	3	104.4	34.8	0.00	**6.55**	
Interaction	**1**	**33.5**	**2.8**	**0.00**	**2.10**	
IPCA1	**6**	**18.8**	**3.1**	**0.00**		**56.12**
IPCA2	**4**	**14.1**	**3.5**	**0.00**		42.09
Residual	2	0.6	0.3	0.56		1.79
Error	4	21.9	0.5		1.37	

FRUIT TSS:TA RATIO

Source of variance	Df	SS	MS	Probability (P≤0.05)	Total Variance	Interaction component
Total	79	418.50	5.30			
Treatment	19	407.10	21.43	0.00		
Environment	4	377.70	94.42	0.00	**90.25**	
Block	15	1.60	0.10	0.94	0.38	
Genotype	3	12.60	4.20	0.00	**3.01**	
Interaction	**12**	**16.80**	**1.40**	**0.00**	**4.01**	
IPCA1	**6**	**15.50**	**2.58**	**0.00**		**92.26**
IPCA2	**4**	**0.80**	**0.21**	**0.44**		4.76
Residual	2	0.50	0.24	0.35		2.98
Error	45	9.80	0.22		2.34	

The model revealed that regarding the quality traits, differences accounted for between the environments (scions), ranged from 89.17% to 97.34% of the total SS for the various traits measured (Table 5.1). Variance accounted for by genotypes (rootstocks) and GEI was also significant and ranged from as little as 0.14% for fruit mass to 6.55% for juice percentage and for GEI ranged from 0.94% for peel thickness to 4.01% for TSS:TA ratio

Cultivar means, ranking of the four rootstocks per environment (citrus scion type) and environmental IPCA scores are presented in Table 5.2. According to Mohammadi *et al*. (2007), environments that contribute little to GEI have IPCA scores close to zero and the closer the scores are to zero the more stable the environment.

Fruit mass: Except for mandarin and grapefruit, all the citrus scion types had IPCA 1 and IPCA 2 scores with the same sign, constituting a proportionate response or non-crosssover GEI. Mandarin had a large positive IPCA1 value (4.40) and IPCA2 score of -1.90 for fruit mass, deeming the mandarins a discriminating but not stable mega-environment for rootstock evaluation.

Peel thickness: Except for mandarin and grapefruit, all the citrus scion types had IPCA 1 and IPCA 2 scores with the same sign, constituting a proportionate response or non-crossover GEI. Grapefruit had a large IPCA1 score of -0.67 (better at discriminating the genotypes) and near zero IPCA2 score of 0.08 (more representative of an average environment).

Juice percentage: Except for Ellendale and midseason, all the citrus scion types had IPCA 1 and IPCA 2 scores with the same sign, constituting a proportionate response or non-crosssover GEI. Ellendale had a large IPCA1 score of -1.28 (better at discriminating the genotypes) and near zero IPCA2 score of 0.12 while midseason had a large IPCA 2 score of -1.18, which demonstrate high instability.

TSS: Except for Valencia and midseason, all the citrus scion types had IPCA 1 and IPCA 2 scores with the same sign, constituting a proportionate response or non-crosssover GEI. Ellendale, midseason and Valencia all had large IPCA 1 scores of 0.40, -0.54 and 0.44 respectively but Ellendale and midseason had a near zero IPCA 2 score.

Table 5.2 Ranking of the four rootstocks (G) for quality aspects mass, peel, juice, TSS, acid and ratio per environment (citrus scion type)

Citrus scion type (E) Environment (ENV)	ENV Mean	IPCA1 Score	IPCA2 Score	Rankings of rootstock genotypes for **Mass (g)** 1	2	3	4
Grapefruit	320.4	-1.38	1.41	306	608	575	42
Ellendale	231.1	0.19	1.59	608	575	306	42
Valencia	230.1	0.27	1.65	608	575	306	42
Mid season	228.7	-3.48	-2.75	306	42	608	575
Mandarin	167.2	4.40	-1.90	42	575	608	306

Environment (ENV)	ENV Mean	IPCA1 Score	IPCA2 Score	Rankings of rootstock genotypes for **Peel** (mm) 1	2	3	4
Grapefruit	7.26	-0.67	0.08	306	608	575	42
Ellendale	3.17	0.24	0.02	608	306	42	575
Valencia	4.86	-0.05	-0.13	306	608	575	42
Mid season	5.18	0.32	0.21	608	42	306	575
Mandarin	3.41	0.15	-0.18	608	306	575	42

Environment (ENV)	ENV Mean	IPCA1 Score	IPCA2 Score	Rankings of rootstock genotypes for **Juice** (%) 1	2	3	4
Grapefruit	47.04	0.56	0.61	608	306	575	42
Ellendale	58.96	-1.28	0.12	306	608	575	42
Valencia	53.27	0.37	0.25	306	608	575	42
Mid season	51.54	0.26	-1.18	306	42	608	575
Mandarin	57.15	0.09	0.19	306	608	575	42

Environment (ENV)	ENV Mean	IPCA1 Score	IPCA2 Score	Rankings of rootstock genotypes for **TSS** (%) 1	2	3	4
Grapefruit	8.91	0.01	0.35	608	42	306	575
Ellendale	12.49	0.40	0.01	608	306	575	42
Valencia	10.75	-0.54	0.31	42	608	306	575
Mid season	11.45	0.44	-0.06	306	608	42	575
Mandarin	9.25	-0.31	-0.60	608	306	575	42

Environment (ENV)	ENV Mean	IPCA1 Score	IPCA2 Score	Rankings of rootstock genotypes for **Acid** (%) 1	2	3	4
Grapefruit	1.21	-0.04	0.02	42	306	575	608
Ellendale	1.13	0.24	-0.10	306	575	608	42
Valencia	1.08	-0.25	-0.11	42	306	575	608
Mid season	0.96	0.12	0.06	42	575	306	608
Mandarin	0.66	-0.07	0.14	306	575	608	42

Environment (ENV)	ENV Mean	IPCA1 Score	IPCA2 Score	Rankings of rootstock genotypes for **Ratio** 1	2	3	4
Grapefruit	8.05	0.32	0.07	608	42	575	306
Ellendale	11.39	0.46	-0.52	608	42	306	575
Valencia	10.41	-0.06	0.07	608	306	42	575
Mid season	12.93	0.47	0.41	608	306	575	42
Mandarin	14.40	-1.20	-0.02	608	575	42	306

Acid: Valencia and midseason had IPCA 1 and IPCA 2 scores with the same sign, constituting a proportionate response or non-crosssover GEI. Grapefruit is a stable environment as it contributed little to GEI by having IPCA scores close to zero. Both Ellendale and Valencia had large IPCA 1 scores and are thus better at discriminating the genotypes (rootstocks).

TSS:TA ratio: All the citrus scion types except Ellendale and Valencia had IPCA 1 and IPCA 2 scores with the same sign, constituting a proportionate response or non-crossover GEI. Valencia is a stable environment as it contributed little to GEI by having IPCA scores close to zero. Mandarin had a large IPCA1 score of -1.2 (better at discriminating the genotypes) and near zero IPCA2 score of -0.02 (more representative of an average environment).

It is thus evident from Table 5.2 that different rootstocks are prominent in different citrus scion types for different attributes and according to Mohammadi *et al.* (2007), large SS for environments as shown in Table 5.1 indicate that the environments (in this case citrus scion types) were diverse, with substantial differences among environmental means causing most of the variation.

Significant GEI and crossover (qualitative) as well as non-crossover (quantitative) interaction for the other citrus scion types concluded that the citrus scion types were indeed mega-environments and Option 2 in Figure 5.1 was executed.

5.3.1 Defining rootstock and scion GEI within citrus scion types

(Figure 5.1: Option 2)
With regard to the characterisation of the stions in this trial, it has been determined that there is a significant interaction amongst the rootstocks and scions (Table 5.1)

To determine the rootstock effect on the scion, the diversity of citrus scion type effect (group effect) was removed and genotypes within a citrus scion type, in this case Ellendale selectons, were thus used to determine the effect of the rootstocks on the scion (Figure 4.3) for each of the fruit quality traits in Table 5.2. Peel thickness was chosen to illustrate rootstock and scion GEI within citrus scion types.

5.3.1.1 Defining stion (G) for peel thickness with regard to years (E)

Prior to investigating the rootstock effect on the scion, the best scion-rootstock combination with regard to peel thickness over years was determined by partitioning the G, E and GEI for stion (G) by years (E) (Table 5.3). The model revealed that differences between the environments (years) albeit significantly accounted for only 0.59% of the treatment SS.

Contribution of the genotypes (stion) and GEI to the total SS respectively accounted significantly for 88.91% and 10.50% of the treatment SS. The SS for the IPCA1 and IPCA2 was significant at P<0.05 and captured 63.90 and 20.75%, respectively.

Table 5.3 AMMI analysis of variance for peel thickness of stion (G) evaluated for five consecutive years (E) within the Ellendale group

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained: Total Variance	Treatment variance	Interaction components
Total	479	270.79	0.57				
Treatment	119	236.31	1.99	0.00			
Environment	4	1.39	0.35	0.00	0.51	0.59	
Block	15	0.61	0.04	0.97	0.23		
Genotype	23	210.1	9.14	0.00	77.59	88.91	
Interaction	**92**	**24.82**	**0.27**	**0.00**	9.17	10.50	
IPCA	**26**	**15.86**	**0.61**	**0.00**			63.90
IPCA	**24**	**5.15**	**0.22**	**0.00**			20.75
Residual	42	3.81	0.09	0.61			15.35
Error	345	33.86	0.10		12.50		
					100.00	100.00	100.00

Environmental means, ranking the first four stions per environment (year) with regard to peel thickness for the five production years and first IPCA component scores of the rootstock cultivars are presented in Table 5.4.

Table 5.4 Mean peel thickness (mm), AMMI scores (ranked in descending IPCA 1 order) and first four Ellendale stions (from AMMI estimates) per environment (year)

Environment (E) Years	E mean	IPCA 1 Score	IPCA 2 Score	1	2	3	4
Yr. 3	3.22	1.24	0.07	1033/306	1033/608	228b/306	228b/608
Yr. 2	3.19	-0.08	-0.26	1033/306	1034/306	1032/608	228b/608
Yr. 4	3.20	-0.21	0.15	1033/306	1033/608	228a/306	228b/608
Yr. 5	3.29	-0.43	0.74	1032/306	1032/608	228a/306	228b/608
Yr. 1	3.13	-0.50	0.07	1033/306	1033/608	228a/306	228b/608

5.3.2 Defining scion (G) for peel thickness with regard to environment (E)

5.3.2.1 Defining scion (G) for peel thickness with regard to rootstock effect on scion within a citrus scion type (Group 1: Ellendale mandarin)

An AMMI ANOVA for peel thickness of six Ellendale selections (G) evaluated on four different rootstocks (E) is presented in Table 5.5 and shows significant effects for genotype, environment and GEI with regard to peel thickness on all four rootstocks. The model revealed that differences between the environments (rootstocks) accounted for 1.40% of the treatment SS. Ellendale selections (G) and GEI accounted significantly for 97.19 and 1.43% of variation, respectively

Table 5.5 AMMI analysis of variance for peel thickness of six Ellendale selections (G) within the Ellendale citrus scion type, evaluated on four different rootstocks (E)

					% Variance explained		
Source of variance	df	SS	MS	Probability (P≤0.05)	Total Variance	Treatment variance	Interaction components
Total	95	42.92	0.45				
Treatment	23	42.02	1.83	0.00			
Environment (Rootstock)	3	0.59	0.20	0.00	**1.37**	1.40	
Block	12	0.20	0.02	0.17	0.47		
Genotype (Ellendale selections)	5	40.84	8.17	0.00	**95.15**	97.19	
Interaction	**15**	**0.60**	**0.04**	**0.00**	**1.40**	1.43	
IPCA1	**7**	**0.33**	**0.05**	**0.00**			**55.00**
IPCA2	**5**	**0.17**	**0.03**	**0.02**			28.33
Residual	3	0.10	0.03	0.05			16.67
Error	60	0.69	0.01		1.61		
					100.00	100.00	100.00

Results from the AMMI analysis also showed that the SS for the first principal component axis (IPCA1) captured 55% of the interaction SS in 47% of the df and was significant at P<0.05. Similarly, the IPCA2 accounted for 28.33% of the variation within 33% of the df and was significant (P≤0.05). The magnitude of the genotype SS was 68 times larger than that of the GEI, which indicates that there were little differences in genotypic response over environment (rootstocks). However, the contribution of GEI in explaining the total variance was significant as well as substantial enough to result in crossovers across rootstocks (E).

The first four AMMI recommended Ellendale genotypes per rootstock and IPCA1 component scores for peel thickness of the Ellendale selections are presented in Table 5.6. Mean peel thickness of the four rootstocks varied from 3.11 to 3.29 mm.

Table 5.6 Ranking of the Ellendale selections per rootstocks (E) for peel thickness (mm)

Environment (E)	E mean	IPCA 1 Score	IPCA 2 Score	1	2	3	4
608	3.11	0.38	0.19	1032	1033	1034	228a
575	3.29	0.13	-0.34	1034	228a	228b	1033
306	3.15	-0.23	0.21	1034	1032	1033	228a
42	3.27	-0.28	-0.06	1034	1032	1033	228a

5.3.2.2 Scion (G) genotypic and environment interaction for peel thickness (Group 1: Ellendale mandarin)

To determine the effect of production years (E) on the scion, the effect of citrus scion diversity was removed and genotypes of one citrus scion type was thus used (Figure 4.3 (Chapter 4). An AMMI ANOVA for peel thickness of six Ellendale selections evaluated in five consecutive years is presented in Table 5.7 and shows significant effects for genotype, environment and GEI for peel thickness over all years.

Table 5.7 AMMI analysis of variance for peel thickness of Ellendale Scion (G) genotypes evaluated for five consecutive years (E)

Source of variance	df	SS	MS	Probability ($P \leq 0.05$)	% Variance explained: Total Variance	Treatment variance	Interaction components
Total	119	56.67	0.48				
Treatment	29	54.92	1.89	0.00			
Environment	4	0.35	0.09	0.00	0.62	0.63	
Block	15	0.15	0.01	0.94	0.26		
Genotype	5	51.05	10.21	0.00	90.08	92.95	
Interaction	**20**	**3.53**	**0.18**	**0.00**	6.23	6.42	
IPCA1	**8**	**2.93**	**0.37**	**0.00**			83.00
IPCA2	**6**	**0.51**	**0.09**	**0.00**			14.45
Residual	6	0.08	0.01	0.69			2.27
Error	75	1.59	0.02		2.81		
					100.00	100.00	100.00

The model revealed that differences between the environments (years) accounted for only 0.63% of the treatment SS. Genotypes (scions) and GEI accounted significantly for 92.95 and 6.42%, respectively. The magnitude of the genotype SS was 14.46 times larger than that of the GEI (Table 5.7), which indicates that there were little differences in genotypic response over environments (rootstocks). However, the contribution of GEI in explaining the total variance was significant as well as substantial enough to result in crossovers across rootstocks (E).

The SS for the first principal component axis (IPCA1) was significant at P<0.05 and captured 83% of the interaction SS in 40% of the df. The second principal component axis (IPCA2) explained a further 14.45% and was significant at P<0.05 within 30% of the df.

The first four AMMI recommended Ellendale genotypes per environment (year) and IPCA1 component scores for peel thickness of the Ellendale selections are presented in Table 5.8. Mean peel thickness of the Ellendale selections (G) varied over five seasons (E) between 3.13 and 3.29 mm.

Table 5.8 Ranking of the Ellendale selections per years (E) for peel thickness (mm)

Environment Year (E)	E mean	IPCA 1 Score	IPCA 2 Score	1	2	3	4
Yr. 1	3.13	0.34	0.02	1033	228b	228a	1032
Yr. 5	3.29	0.23	0.44	228a	1033	1034	1032
Yr. 4	3.22	0.17	-0.38	1034	1032	228a	1033
Yr. 2	3.19	0.07	-0.13	1033	1032	1034	228b
Yr. 3	3.20	-0.81	0.04	1033	228b	1032	1034

5.3.3 Defining rootstock (G) for peel thickness with regard to environment (E)

5.3.3.1 Defining rootstock (G) for peel thickness with regard to scion effect on rootstock (Group 1: Ellendale mandarin)

To determine the scion effect on the rootstock, the diversity due to citrus scion type (group effect) was removed and genotypes within a group were thus used (Figure 4.4). An AMMI ANOVA for peel thickness of four rootstock genotypes budded with six Ellendale selections evaluated in five consecutive years is presented in Table 5.9. It showed that there were significant effects for genotype and environment but not for GEI with regard to peel thickness.

The model revealed that differences between the Ellendale selections (E) accounted for 97.52% of the treatment SS. Contribution of the Rootstocks (G) to the treatment SS accounted significantly for 1.34% while a GEI of 1.17% was non-significant. The SS for the IPCA1, IPCA2 and the residual were also non-significant at P≤0.05.

The large SS for Ellendale selections (E) indicated the diversity of the Ellendale selections, with large differences among environmental means causing most of the variation in peel thickness

Table 5.9 AMMI analysis of variance for peel thickness of four rootstocks (G) genotypes evaluated with six Ellendale selections (E) within the Ellendale group

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained: Total Variance	Treatment variance	Interaction components
Total	95	42.26	0.45				
Treatment	23	41.88	1.82	0.00			
Environment (Ellendale selections)	5	40.84	8.17	0.00	96.64	97.52	
Block	18	0.13	0.01	1.00	0.31		
Genotype (Rootstocks)	3	0.56	0.19	0.05	1.33	1.34	
Interaction	**15**	**0.49**	**0.03**	**0.67**	1.16	1.17	
IPCA	**7**	**0.27**	**0.04**	**0.53**			55.10
IPCA	**5**	**0.14**	**0.03**	**0.67**			28.57
Residual	3	0.08	0.03	0.62			16.33
Error	6	0.25	0.04		0.59		
					100.00		100.00

Environmental means, ranking of the four rootstocks per Ellendale selctions (E) and first IPCA component scores of the rootstocks (G) are presented in Table 5.10. Mean peel thickness of the six environments varied from 2.84 to 4.66 mm.

Table 5.10 Ranking of the rootstocks (G) per environment (Ellendale selections) for peel thickness (mm)

Environment (E) Ellendale selections	Env. mean	IPCA 1 Score	IPCA 2 Score	1	2	3	4
228b	3.02	0.18	-0.23	306	42	608	575
1034	2.84	0.13	0.02	306	608	42	575
228a	2.95	0.10	-0.17	306	608	42	575
1033	2.89	0.04	0.20	608	306	42	575
1032	2.88	0.03	0.27	608	306	42	575
1085	4.66	-0.48	-0.09	608	575	306	42

5.3.3.2 Rootstock (G) genotypic and environment interaction for peel thickness (Group 1: Ellendale mandarin)

In order to determine the environment effect on the rootstock, the diversity due to citrus scion type (group effect) was removed and genotypes within a group were thus used (see Figure 4.4). An AMMI analysis for peel thickness of four rootstocks (G) evaluated with Ellendale selections for five years (E), is presented in Table 5.11 and showed that the rootstocks (G) and production years (E) were significant (P≤0.05) but GEI with regard to peel thickness in this instance was not significant.

Table 5.11 AMMI analysis of variance for peel thickness of four rootstocks (G) genotypes evaluated for five consecutive years (E) within the Ellendale group

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained: Total Variance	Treatment variance	Interaction components
Total	79	1.80	0.02				
Treatment	19	0.98	0.05	0.00			
Environment (Year)	4	0.23	0.06	0.00	12.92	23.59	
Block	15	0.10	0.01	0.96	5.68		
Genotype (Rootstocks)	3	0.49	0.16	0.00	27.22	49.67	
Interaction	**12**	**0.26**	**0.02**	**0.21**	14.65	26.74	
IPCA	**6**	**0.15**	**0.03**	**0.17**			57.46
IPCA	**4**	**0.10**	**0.03**	**0.18**			39.23
Residual	2	0.01	0.00	0.76			3.30
Error	45	0.71	0.02		39.53		
					100.00		100.00

The model revealed that differences between the production years (E) accounted for a significant percentage (23.59%) of the treatment SS. Contribution of the rootstocks (G) to the treatment SS accounted significantly for 49.67% while a GEI of 26.74% was found to be non-significant. The SS for the IPCA1, IPCA2 and residual were also non-significant at P≤0.05. The means of peel thickness for each year, ranking of the four rootstocks per year (E) and first IPCA component scores of the rootstock cultivars are presented in Table 5.12.

Table 5.12 Ranking of the rootstock genotypes per production year (E) for peel thickness (mm)

Environment Year (E)	E mean	IPCA 1 Score	IPCA 2 Score	1	2	3	4
Yr. 5	3.29	0.21	0.00	608	306	575	42
Yr. 3	3.22	0.14	0.06	608	306	42	575
Yr. 2	3.19	0.01	-0.32	306	608	575	42
Yr. 4	3.20	0.00	0.24	608	306	42	575
Yr. 1	3.13	-0.36	0.02	42	306	608	575

5.3.4 Summary of the complex genotype x environment relationship of a grafted tree partitioned into G, E and GEI regarding peel thickness within each of four citrus citrus scion types as per AMMI analysis.

The information in Tables 5.3 through to 5.12 pertains to G, E and GEI within the Ellendale group for peel thickness. Figure 5.4 depicts G, E and GEI for peel thickness of a grafted Ellendale tree where interaction between a rootstock and scion is disregarded and the observed GEI is environment interaction with the combined genotype (stion).

Figure 5.3 illustrates the simplified stion x environment relationship of a grafted tree while Figure 5.4 depicts the complex genotype x environment relationship of a grafted tree partitioned into G, E and GEI for peel thickness within the group Ellendale.

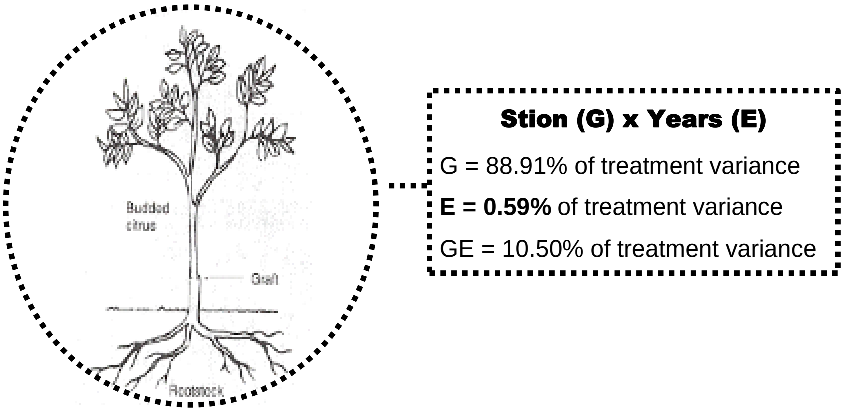

Figure 5.3 Summary of the simplified stion x environment context of a grafted tree partitioned into G, E and GEI regarding peel thickness within the group Ellendale but disregarding specific interactions pertaining to rootstock and scion

Figure 5.4 depicts the complex genotype x environment context of a grafted tree partitioned with regard to scion and rootstock into G, E and GEI within the group Data

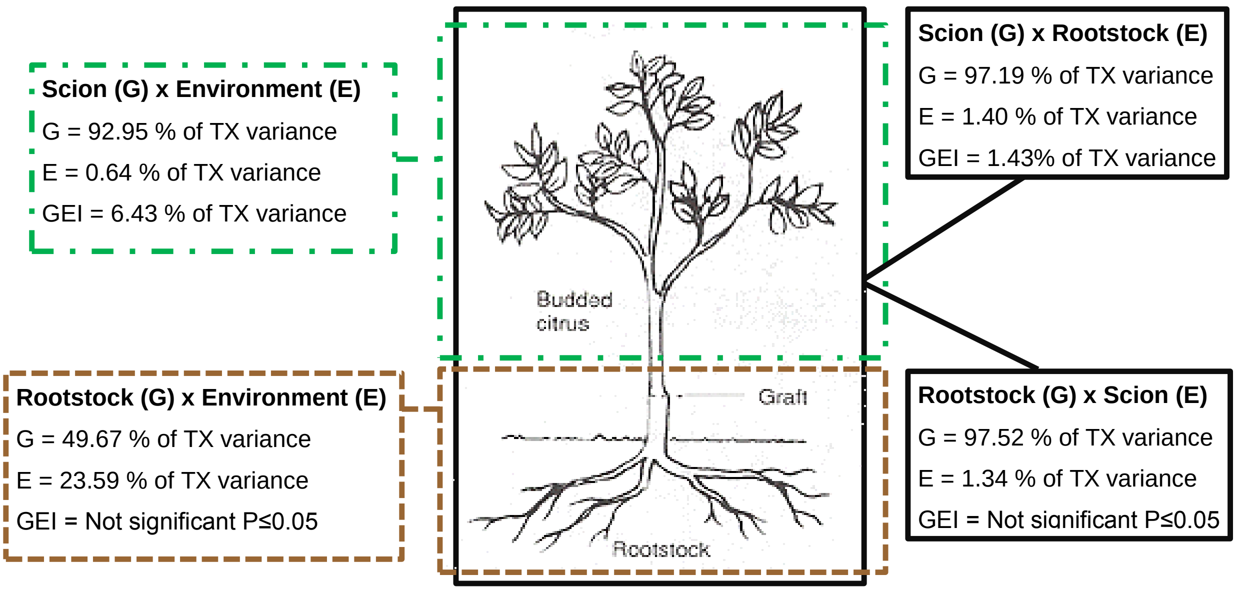

Figure 5.4 Summary of the complex genotype x environment context of a grafted tree partitioned into G, E and GEI regarding peel thickness within the citrus scion type Ellendale (TX = treatment)

Data was obtained from the AMMI ANOVA Tables 5.3 through to 5.12. The same reasoning and procedure was followed for mandarin, grapefruit and Valencia and the resulting data are summarised in Table 5.13.

Table 5.13 Summary and comparison of variance percentages of the treatment SS for peel thickness accounted for by G, E and GEI within each of four citrus citrus scion types grafted to four different rootstocks and evaluated over five years in one locality as per AMMI analysis

****Source of variance**	***Stion (G) x Years (E)**	**Scion (G) x Rootstock (E)**	**Rootstock (G) x Scion (E)**	**Scion (G) x Years (E)**	**Rootstock (G) x Years (E)**
	% of the treatment variance explained**				
			Ellendale		
Genotypes	88.91	97.19	1.34	92.95	49.67
Environments	0.59	1.40	97.52	0.64	23.59
Interactions	10.50	1.43	NS	6.43	NS
IPCA1	63.90	55.00	NS	83.00	NS
IPCA2	20.75	28.33	NS	14.45	NS
			Mandarin		
Genotypes	13.54	29.85	14.62	6.01	3.77
Environments	45.96	14.62	29.85	68.36	87.44
Interactions	40.50	55.52	55.52	25.63	8.79
IPCA1	57.73	80.80	80.80	81.30	87.68
IPCA2	23.50	18.84	18.84	13.55	NS
			Grapefruit		
Genotypes	35.15	38.23	54.96	20.27	30.41
Environments	40.74	54.96	38.23	61.44	64.11
Interactions	24.10	NS	NS	18.30	5.49
IPCA1	42.16	NS	NS	67.91	82.46
IPCA2	40.07	NS	NS	20.71	NS
			Valencia		
Genotypes	38.32	16.06	59.75	14.49	47.51
Environments	17.49	59.75	16.06	41.19	36.29
Interactions	44.20	24.21	24.21	44.34	16.18
IPCA1	43.25	56.86	56.86	67.54	61.23
IPCA2	25.19	NS	NS	16.67	24.97

* Residual values for mandarin and Valencia were significant and a very high residual percentage was evident for Valencia for this option.
** The IPCA percentages per column will not add to 100% as the residual were not included in this table.

Ellendale: The AMMI ANOVA for peel thickness of the stion combinations within the Ellendale group tested over five years showed that genotypes and environment main effects and GEI were significant (P≤0.05); suggesting that GEI influenced the performance of the stions with regard to peel thickness in all years. These results showed that 88.91% of the treatment SS was attributable to the genotype. Variation regarding peel thickness with regard to stion (G) x year (E), was largely attributable to the genotype (88.91%) while GEI and environment effects respectively accounted for 10.50% and 0.59% of the variation (Table 5.13).

By partitioning of the scion and the rootstock it was found that 92.95% of the variation observed were attributable to the scion and only 0.64% to the production years (E) and a significant portion of 6.43% was due to GEI amongst the scion and the years. The rootstock genotypes on the other hand attributed only to 49.67% of the variation within a stion while 23.59% were due to the production years (E). However, in the case of the rootstock no significant GEI was detected amongst rootstocks and production years (E).

When considering the contribution of the scion and rootstock to the phenotype of peel thickness, it was found the 97.19 and 97.52% of the phenotype was attributable to the scion depending on whether it was deemed genotypes within an environment of rootstocks or as environments for rootstock types respectively. Correspondingly, the rootstocks accounted for 1.34% and 1.40% of variation depending on whether it was deemed genotypes within an environment of scions or as environments for scion selections respectively.

*Mandarins: V*ariation accounted for with regard to peel thickness within a stion combination, within the mandarin group was largely attributable to the environment (45.96%) and the interaction of the stion with the years (E) (40.50%). While the genotype of the stion only accounted for 13.54% (Table 5.13). The AMMI ANOVA for peel thickness of the stion combinations tested over five years showed that genotypes and environment main effects and GEI were significant ($P \leq 0.05$); suggesting that GEI influenced the performance of the stions with regard to peel thickness in all years.

By partitioning of the scion and the rootstock it was found that only 6.01% of the variation observed was attributable to the scion and 68.36% to the production years (E). The rootstock genotypes on the other hand contributed only to 3.77% of the variation within a stion while 87.44% was due to the production years (E). However, variation accounted for due to GEI amongst the scion and the years was 25.63% while GEI amongst the rootstock and the years was only 8.79%. This indicates that the scions were much more sensitive to the production years (E) than the rootstocks as is illustrated by the rankings of the top four genotypes per production years (E) with regard to scion and rootstock in Table 5.14.

When considering the contribution of the scion and rootstock to the phenotype of peel thickness, it was found that 29.85 and 14.62% of the phenotype was attributable to the Mandarin scion type and the rootstock, respectively. However, a significant GEI of 55.52% contributed substantially to the variation thus depending on the interactions or the differential response of scions to rootstocks and *vice versa*, the ranking of best scion per rootstock will also differ greatly amongst scions as will the most suitable rootstock per scion amongst rootstocks. This is illustrated in Table 5.14 where rootstock 575 ranked first for mandarin selection 918 but only third for selections 1121 and 803a. Correspondingly mandarin selection 803a ranked first on rootstock 306 but only fourth on 575 and 42.

Grapefruit: The most interesting results as in Chapter 4 pertain to the grapefruit group. Variation accounted for regarding peel thickness with regard to stion (G) x year (E), within the grapefruit group was within the same magnitude for the genotype (35.15%) and the production years (E) (40.74%) while the GEI value accounted for 24.10% of the treatment SS (Table 5.13).

The AMMI model revealed that differences between the scions and rootstocks accounted for respectively 38.23 and 54.96% of the treatment SS while the GEI was non-significant. Partitioning of the scion and the rootstock further revealed that 20.27% of the variation observed was attributable to the scion genotypes and 61.44% to production years (E). The rootstock genotypes on the other hand contributed 30.41% of the variation within a stion while 64.11% was due to the production years (E). GEI amongst the scion and the years accounted for a significant portion of 18.30% while GEI amongst the rootstocks and the years accounted for only 5.49% of variation.

Valencia: Variation accounted for (calculated from the treatment SS) with regard to stion (G) x year (E), concerning peel thickness within the Valencia group was largely attributable to the genotype (38.32%) and GEI (44.20%) while the years (E) accounted for 17.49% of the treatment SS values (Table 5.13). The AMMI model revealed that differences between the scions and rootstocks accounted for respectively 16.06 and 59.75% of the treatment SS while the GEI accounted for 24.21% of the treatment SS values (Table 5.13). Partitioning of the scion and the rootstock further revealed that 14.49% of the variation observed was attributable to the scion genotypes while the variance accounted for by the years (E) and GEI amongst the scion and the years were within same magnitude namely

41.19 and 44.34%. The rootstock genotypes on the other hand contributed 47.51% of the variation within a stion while 36.29 and 16.18% were respectively due to the environment and the GEI amongst the rootstocks and the years.

Genotype recommendation

In each environment, AMMI selected the best genotypes that were suitable and adapted for that locality. Table 5.14 shows a comparison of the top four genotypes (G) as determined by AMMI for mean peel thickness per environment (E) for the partitioning of the rootstock and scion effects per citrus scion types Ellendale, mandarin, grapefruit, and Valencia. According to Fox *et al*. (1990) a genotype usualy found in the top third of the entries across environments can be considered relatively well adapted.

Ellendale: Ellendale selections 1032, 1033 and 1034 appeared in the top third of the entries in all of the environments (rootstocks and years) but 1034 were dominant in four of nine environments (rootstocks and years) and 1033 were dominant in three of the nine environments.

Rootstocks for Ellendale: From the rankings of the top four genotypes (G) for peel thickness per environment (E) for the Ellendale group in in Table 5.14 it was evident that rootstocks 306 and 608 were the top performing rootstocks, as it manifested in the top third of entries across environments both for years and scion selections.

Mandarin: Mandarin selections 803a, 918 and 1211 appeared in the top third of the entries in all of the environments (rootstocks and years) but 918 were dominant in five of nine environments (rootstocks and years) and 1211 were dominant in three of the nine environments.

Rootstocks for mandarin: From the rankings of the top four genotypes (G) for peel thickness per environment (E) for the Ellendale group in in Table 5.14 it was evident that rootstocks 42 were the top performing rootstock, as it were dominant in six of the nine environments (years and scion selections).

Table 5.14 Comparison of the rankings of the top four genotypes (G) for peelthickness per environment (E) for rootstock and scion effects per citrus scion types Ellendale, mandarin, grapefruit, and Valencia

Ellendale

ENV	Mean	1	2	3	4	ENV	Mean	1	2	3	4
Rootstock (G) x Scion (E)						Scion (G) x Rootstock (E)					
1034	2.84	306	608	42	575	608	3.11	1032	1033	1034	228a
1032	2.88	608	306	42	575	306	3.15	1034	1032	1033	228a
1033	2.89	608	306	42	575	42	3.27	1034	1032	1033	228a
228a	2.95	306	608	42	575	575	3.29	1034	228a	228b	1033
228b	3.02	306	42	608	575						
1085	4.66	608	575	306	42						
Rootstock (G) x Year (E)						Scion (G) x Year (E)					
Yr. 1	3.13	42	306	608	575	Yr. 1	3.13	1033	228b	228a	1032
Yr. 2	3.19	306	608	575	42	Yr. 2	3.19	1033	1032	1034	228b
Yr. 3	3.22	608	306	42	575	Yr. 3	3.22	1034	1032	228a	1033
Yr. 4	3.20	608	306	42	575	Yr. 4	3.20	1033	228b	1032	1034
Yr. 5	3.29	608	306	42	575	Yr. 5	3.29	228a	1033	1034	1032

Mandarin

Rootstock (G) x Scion (E)						Scion (G) x Rootstock (E)					
918	3.23	575	42	608	306	608	3.22	1211	803a	918	1082
1211	3.25	608	42	575	306	306	3.36	803a	918	1211	1082
803a	3.42	306	608	575	42	575	3.38	918	1211	1082	803a
1082	3.45	42	575	608	306	42	3.40	918	1211	1082	803a
Rootstock (G) x Year (E)						Scion (G) x Year (E)					
Yr. 1	3.03	42	306	575	608	Yr. 1	3.03	918	1211	1082	803a
Yr. 2	3.88	42	306	575	608	Yr. 2	3.88	1211	803a	918	1082
Yr. 3	3.56	42	575	306	608	Yr. 3	3.56	918	1082	803a	1211
Yr. 4	3.23	42	575	306	608	Yr. 4	3.23	1211	803a	918	1082
Yr. 5	3.00	42	575	608	306	Yr. 5	3.00	918	1211	1082	803a

Grapefruit

Rootstock (G) x Scion (E)						Scion (G) x Rootstock (E)					
1179	6.50	608	306	42	575	306	6.73	1179	1183	1057a	231
1183	6.82	306	608	42	575	608	6.98	1179	1183	1057b	1057a
1057b	7.23	306	608	575	42	575	7.61	1179	1183	1057b	1057a
1058	7.47	306	608	575	42	42	7.82	1179	1183	1057b	1057a
1057a	7.25	306	608	575	42						
1176	7.57	306	608	575	42						
1053	7.69	306	608	575	42						
1069	7.61	306	608	575	42						
231	7.42	306	608	575	42						
Rootstock (G) x Year (E)						Scion (G) x Year (E)					
Yr. 1	8.43	306	608	42	575	Yr. 1	8.43	1179	1183	1057b	1057a
Yr. 2	7.22	608	306	575	42	Yr. 2	7.22	1183	1179	1057a	1057b
Yr. 3	7.30	306	608	42	575	Yr. 3	7.30	1179	1183	1057b	231
Yr. 4	7.01	306	608	575	42	Yr. 4	7.01	231	1057a	1179	1057b
Yr. 5	6.46	306	608	575	42	Yr. 5	6.46	1179	231	1183	1057b

Valencia

Rootstock (G) x Scion (E)						Scion (G) x Rootstock (E)					
1060	4.65	608	306	575	42	608	4.67	1060	1055	1056	1052
1052	4.79	608	306	575	42	306	4.68	1043b	1060	1044	1063
1063	4.85	608	306	575	42	575	5.01	1060	1052	1055	1063
1055	4.85	608	306	575	42	42	5.06	1060	1044	1052	1063
1044	4.88	306	42	608	575						
1056	4.89	608	306	575	42						
1043b	4.93	306	608	42	575						
1043a	4.98	306	608	42	575						
Rootstock (G) x Year (E)						Scion (G) x Year (E)					
Yr. 1	5.15	608	306	575	42	Yr. 1	5.15	1044	1060	1052	1055
Yr. 2	4.83	608	306	42	575	Yr. 2	4.83	1044	1060	1052	1055
Yr. 3	4.84	608	306	42	575	Yr. 3	4.84	1060	1052	1055	1063
Yr. 4	4.70	608	306	575	42	Yr. 4	4.70	1060	1063	1055	1056
Yr. 5	4.75	608	306	575	42	Yr. 5	4.75	1060	1063	1055	1056

Grapefruit: Grapefruit selections 1179, 1183 and 231 appeared in the top third of the entries in all of the environments (rootstocks and years) but 11 were dominant in seven of nine environments (rootstocks and years) and 1183 were dominant in three of the nine environments.

Rootstocks for grapefruit: From the rankings of the top four genotypes (G) for peel thickness per environment (E) for the grapefruit group in in Table 5.14 it was evident that rootstocks 306 and 608 were the top performing rootstocks, as it manifested in the top third of entries across environments both for years and scion selections but 306 were dominant in 12 of the 14 environments.

Valencia: Valencia selections 1060 and 1044 appeared in the top third of the entries in all of the environments (rootstocks and years) but 1060 were dominant in six of nine environments (rootstocks and years) and 1044 were dominant in three of the nine environments.

Rootstocks for Valencia: From the rankings of the top four genotypes (G) for peel thickness per environment (E) for the grapefruit group in in Table 5.14 it was evident that rootstocks 306 and 608 were the top performing rootstocks, as it manifested in the top third of entries across environments both for years and scion selections with 608 dominant in 10 of the 13 environments.

5.4 DISCUSSION

The objective of this study was to determine whether it is possible to partition the reaction of the stion systematically into scion and rootstock reactions with regard to quality aspects, in particular peel thickness.

The combination of two different genotypes in a single composite plant to solve a variety of horticultural problems is an age-old practice (Mudge *et al*., 2009). This way the improvement of traits bypasses the reproductive cycle thus eliminating years of selection by means of breeding and rather becomes the result of cellular or genetic interactions in the somatic cells. Koepke and Dhingra (2013) assigned the term 'somatogenetic interactions' to represent this phenomenon in composite plants. Somatogenetic interactions can cause instant physiological modification of desirable traits in the scion on the same level that genetic changes would have but that is rather orchestrated by several hypothesized agents derived from the rootstock (Koepke and Dhingra, 2013). It is thus obvious that combining genotypes in a single composite plant (stion) complicates the determination of heritability of traits as well as the determination of a genotype's stability and adaptability.

Gauch and Zobel (1996) declared noise and interaction amongst the genotypes and environments to be the fundamental problems to be solved in any trial aimed at selecting superior genotypes in a breeding programme or at recommending new cultivars to farmers. AMMI was found to address both of these problems. Noise can be countered by sound trial layouts and management. With regard to GEI two approaches exits, one aimed at genotypes by seeking widely adapted genotypes and the other at environments by identifying homogenous macro environments and then breed and recommend cultivars specifically for these environments. Due to somagenetic interactions AMMI analysis was incorporated in the approach in Figure 5.1 to solve noise and interaction amongst the genotypes and environments.

5.4.1 Defining rootstock (G) with regard to citrus scion type effect on rootstock

The questions in Figure 5.1 (Option 1) pertain to whether the five very different citrus scion types were indeed mega-environments and whether there were any significant and important crossovers.

Test of environment: The large SS for environments (in this case citrus scion types) indicated that the environments (citrus scion types) were diverse, with large differences among environmental means causing most of the variation for the quality attributes. This was to be expected as the environments, namely the citrus scion types, are very diverse by nature, ranging from a small mandarin to a large grapefruit. For instance, regarding fruit mass the magnitude of the GEI interaction SS was 21.39 times larger than that of the genotype, indicating that there were sustainable differences in genotype response across environments. The first question pertaining to whether there is any GEI with regard to rootstock grafted to very different citrus scion types it was found that GEI was significant and that rootstock evaluation should be specific to each mega-environment (citrus scion type).

Rootstock genotype recommendations: According to Fox *et al.,* (1990) a genotype usually found in the top third of entries across environments can be considered relatively well adapted for the trait investigated. Figure 5.5 summarises the percentage of times that a rootstock emerged as the top ranking performer over attributes per citrus scion type. It can be seen that rootstock 575 did not play a significant role in any of the citrus scion types and 608 was the most prominent rootstock with regard to quality attributes.

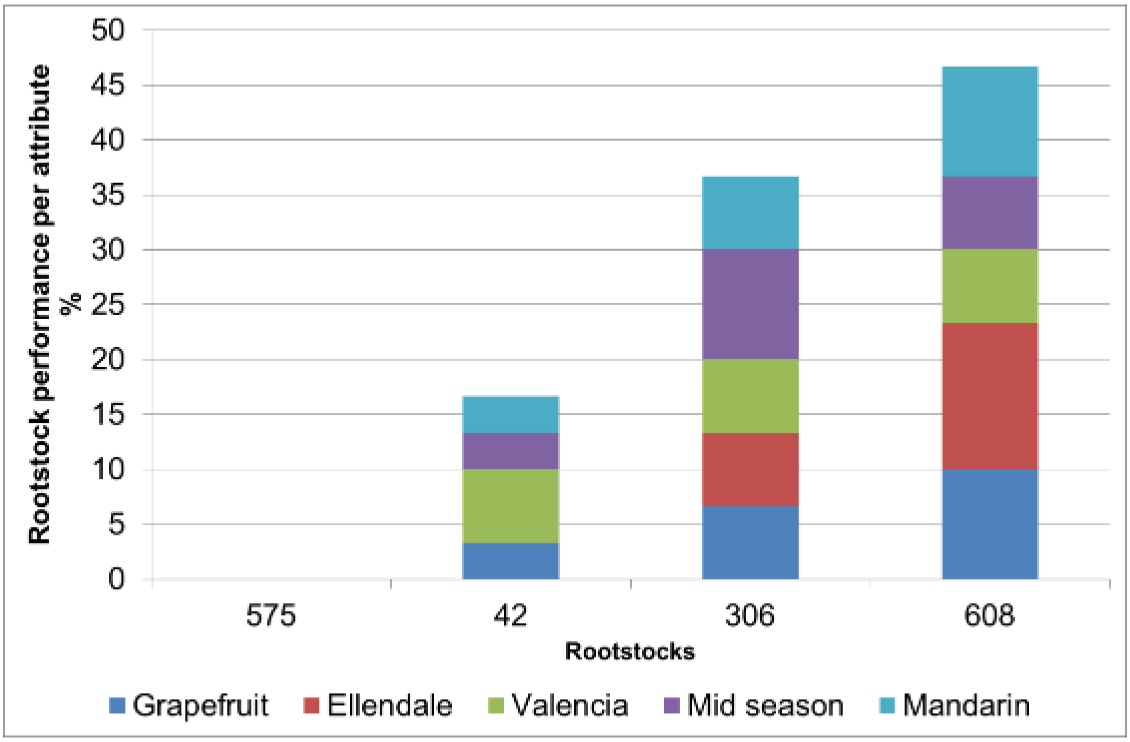

Figure 5.5 Percentage of times that a rootstock emerges as the top ranking performer in an attribute per citrus scion type

Figure 5.6 illustrates the percentage of times that a rootstock emerged as the top ranking performer in a citrus scion type, per attribute. Rootstock 42 did not feature as a top ranking rootstock in any of the citrus scion types with regard to juice percentage, peel thickness and TSS:TA ratio and 608 was the top ranking rootstock in all the citrus scion types with regard to TSS:TA ratio.

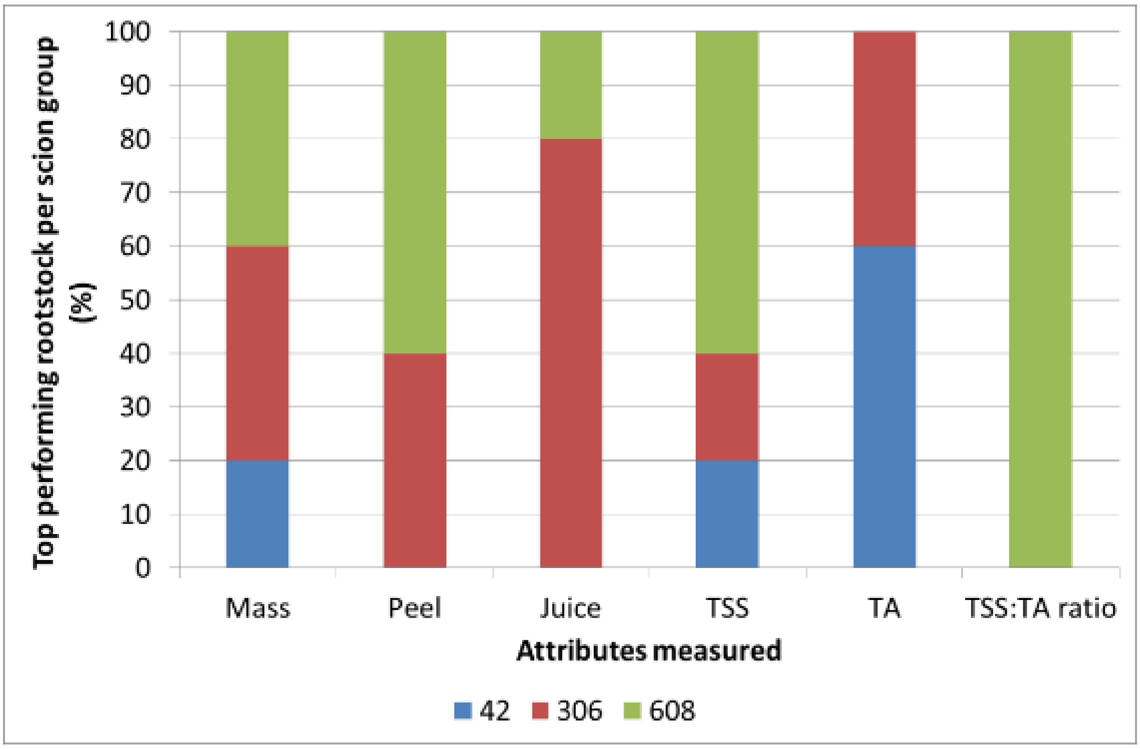

Figure 5.6 Percentage of times that a rootstock emerges as the top ranking performer in a citrus scion type, per attribute

With regard to the second question whether there is important crossover GEI, the contribution to total variance of both the genotype and the GEI were highly significant and within the same range. The GEI was large enough to cause crossovers, thus indicating that there were differences in genotypic (rootstock) response over environments (citrus scion type) warranting further investigation (Figure 5.1 Option 2).

Citrus scion types as mega-environment per attribute

- According to Farshadfar *et al*. (2011), a genotype/environment that has a large positive IPCA score with some environments/genotypes must have negative interaction with some other environments/genotypes.
- In contrast, a proportionate genotype response or a non-crossover (quantitative) GEI will be represented by scores with the same sign or near zero (Mohammadi and Amri, 2008; Farshadfar *et al.*, 2011). Genotypes and environments with IPCA 1 scores of zero or near zero have small interaction (Crossa, 1990).

- Relating to mega-environments, ideal test environments should have large IPCA1 scores (better at discriminating the genotypes) and near zero IPCA2 scores (more representative of an average environment) (Yan, 1999; Yan *et al*., 2000).

Based on the IPCA values only mandarin was highly discriminative, but unstable with regard to rootstock evaluation for fruit mass. With regard to peel thickness and juice percentage, grapefruit and Ellendale respectively represented the ideal test environment with large IPCA1 scores and IPCA2 scores close to zero. The best mega-environments for rootstocks regarding TSS were Ellendale and midseason oranges, but Ellendale was more stable. For acid, the grapefruit was the stable environment for evaluating rootstock performance while Ellendale and Valencia were more discriminating but not as stable. With regard to TSS:TA ratio, the mandarin group constituted a discriminating and stable environment for rootstock evaluation while Valencia was the most stable environment with regard to ratio as it contributed little to GEI by having IPCA scores close to zero.

Significant GEI and crossover (qualitative) as well as non-crossover (quantitative) interaction for the other citrus scion types concluded that the citrus scion types were indeed mega-environments and Option 2 in Figure 5.1 was executed.

5.4.2 Separating the stion GEI into a scion GEI and a rootstock GEI for peel thickness per mega-environment (citrus scion type)

Peel thickness requirements are market dependent. With regard to the market demands on fresh fruit, thicker peels offer less damage due to pests and diseases as well as a better shelf life and in soft citrus such as mandarin and Ellendale thicker fruit are perceived as easier to peel. However, grapefruit and oranges with tight adhering peels are normally prone to excessively thick peels that are undesirable. With regard to processing, thicker peels lead to a lower juice production but higher levels of oil for extraction from the peel.

The objective was to separate and quantify the contribution of the scion and rootstock to the stion in a single physical environment (i.e same climate, same soil, same production practices). This was found to be possible as illustrated in Table 5.13. The importance of this is threefold:

i. more effective selection of genotypes, either rootstocks or scions, based on G and GEI rather than the phenotype of the stion and

ii. quantify, determine and allocate the causes of GEI to the appropriate part of the scion
iii. mitigating or exploiting the impact of environmental influences through genotype combination and/or manipulation of the environment through horticultural practices.

The question in option 2 of Figure 5.1 probed into whether there was any GEI pertaining to scion and rootstock within a citrus scion type.

Grapefruit as mega environment for peel thickness: In option 1 of Figure 5.1 grapefruit represented the ideal test environment for peel thickness with large IPCA1 scores and IPCA2 scores close to zero. With regard to grapefruit selections, no significant GEI amongst scions and rootstocks regarding peel thickness proved it a simple mega-environment without any crossovers, signifying that comparison of grapefruit selections can be done on any rootstock. This implies that the same grapefruit selection will be identified as the best selection no matter the rootstock used. However, the rootstock contribution to the phenotype was significant and impacts on the magnitude of the observation, comparison of selections should thus only be done on a single rootstock and never be compared over rootstocks.

Ellendale as mega environment for peel thickness: Concerning the influence of scion and rootstock interactions on the peel phenotype of Ellendale, a small but significant GEI was found amongst Ellendale scions (G) and the rootstocks (E) in this trial. In contrast, there was no significant interaction amongst the rootstocks (G) and the scions (E), which is illuminated by the fact that the GEI amongst scion and years were significant while the interaction of the rootstock with the environment in this case were not significant. The peel phenotype of the Ellendale selections is thus attributable to the scion and the environment affecting the scion.

Mandarins as mega environment for peel thickness: Separation and quantification of the scion and rootstock contributions in mandarins revealed that the peel phenotype of mandarins was predominantly determined by the rootstock (54.96%) as well as the differential response of the mandarin scions to the year effects. GEI is present but mandarins are neither a discriminative nor a representative environment for rootstock selection. However, it still constitutes a relative simple ME but evaluation of mandarin selections should be conducted on a specific rootstock

Valencia as mega environment for peel thickness: As in grapefruit, the peel phenotype of the Valencia stion was also predominantly determined by the rootstock (59.75%). However, a large GEI between scions and rootstocks signify that Valencia is a complex ME and thus not the best test environment for rootstocks. Rootstocks for this group and selections within this group should be selected on both mean and stability and not just on the highest mean. When genotypic main effects were the only consideration (Chapter 3) new Valencia selections 1044, 1055, 1060, 1062 and 1063 were found not to differ significantly. Following the AMMI procedure considering GEI, that included rootstock scion interactions, it was found that selections 1060 appeared in the top third of the entries in all of the environments (rootstocks and years).

5.5 CONCLUSION

Development of cultivars with high and stable genetic potential for quality is one of the main goals of a fruit-breeding programme. However, fruit quality in itself was found to be complex, comprising an interchange of molecular, physiological, and biochemical processes let alone the complications expected from a citrus tree being the combination of two different but compatible genotypes merged by budding to form a compound genetic unit.

The influence of rootstocks as well as climate on fruit quality in citrus is thus the reason for genetic material (newly bred cultivars) to be assessed on different rootstocks and over a number of years for a certain area and even sometimes in more than one locality (multi-environment trials).

When assessing new rootstock selections, an "ideal" test environment should be both discriminating of the genotypes and representative of the mega-environment and/or target environment.

Regarding the first question pertaining to whether there is any GEI with regard to rootstock grafted to very different citrus scion types, it was found that GEI was significant and that rootstock evaluation should be specific to each mega-environment (citrus scion type). No single mega environment for rootstock selection, that was both discriminative and stable with regard to all the traits in this trial, could be identified. Mega environments were thus trait specific with, for instance grapefruit, representing the ideal test environment for peel thickness and Valencia was the most stable environment with regard to TSS:TA ratio. If the only objective in rootstock breeding was to find a new rootstock that positively modifies peel thickness, grapefruit would have been the choice of cultivar for testing the rootstocks.

By assessing the GE data for the traits within a single mega-environment, it was determined that stion GEI can successfully be separated into a scion GEI and a rootstock GEI for different quality traits per mega-environment (citrus scion type). No significant interaction amongst the scion and rootstocks (Table 5.13) echoed the findings of grapefruit being a mega environment with regard to peel thickness.

GEI amongst scions and rootstocks can have either a positive or a negative influence on the trait being investigated. The existence of GEI complicates the identification of superior genotypes for a range of environments. Yan and Tinker (2006) accentuate the facts that whenever there is significant GEI, the potential causes should be explored in order to mitigate or exploit the GEI. However, genetic and environmental covariates are necessary to address this matter.

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Chapter 6

AMMI AND GGE BIPLOTS AS A GRAPHICAL TOOL FOR GEI ANALYSIS REGARDING CITRUS SCIONS AND ROOTSTOCKS

Abstract

Questions typically asked by producers in horticulture relate to which cultivar will perform best in their climate/soil, which rootstock is most disease-resistant or which cultivar has the highest yield? Normally a number of genotypes is tested over a number of sites and years to evaluate adaptation of the crop or to select the best genotype per environment and it is often difficult to determine the pattern of genotypic responses across environments without the use of appropriate analytical and statistical tools. Another complicating matter in determining the pattern of genotypic responses across environments, is the differential genotypic expression across environments (GEI). If GEI exists, means across trials are of limited use for meaningful recommendations. Understanding the causes of GEI are useful for establishing breeding objectives, identifying ideal test environments and to formulate recommendations with regard to optimal genotype adaptation to areas. A model that clearly differentiates between the main and interaction effects is thus needed. Additive main effects and multiplicative interactions (AMMI) and site regression (SREG) genotype plus genotype-by-environment interaction (GGE) models are the current models that effectively capture the additive (linear) and multiplicative (bilinear) components of GEI and provide meaningful interpretation of multi-environment data set in breeding programs.

GGE is an acronym for genotype main effect (G) plus GEI, which is the only source of variation that is relevant to cultivar evaluation. Mathematically, GGE is the G by E data matrix after the environment means are subtracted and the GGE biplot is consequently the visualisation tool, which graphically displays a GEI in a two-way table. Data were taken from the same trial site and years as discussed in Chapter 4. The propagation methods, orchard practices, trial layout, scions and rootstock genotypes were thus the same. For the purpose of illustration of the AMMI and GGE biplots the same data for mega environment identification and the same subset of data for genotype and test environment evaluation were used. It was found that GGE biplots and the biplot function of the AMMI model provided a more complete and visual evaluation of all aspects of the data. The method is fast and accurate when applying the easy interpretation rules to the biplots which can simultaneously both exhibit mean performance and stability within a mega environment. By applying the easy interpretation rules, visual analysis with the AMMI and GGE biplot was relatively simple, fast and accurate and was found to be beneficial in identifying mega-environments, estimating the magnitude and significance of GEI and to determine general and specific adaptability of genotypes in relation to the test environments.

6.1 INTRODUCTION

Questions typically asked by producers in horticulture relate to which cultivar will perform best in their climate/soil, which rootstock is most disease-resistant or which cultivar has the highest crop yield? In some horticultural trials, only one rootstock with a number of scions is used, as the study pertains to the treatment effect such as, for instance, the effect of pruning on yield. However, normally a number of genotypes is tested over a number of sites and years to see adaptation of the crop or to select the best genotype per environment and it is often difficult to determine the pattern of genotypic responses across environments without the use of appropriate analytical and statistical tools (Yan, 2001). Not only does data from these trials consist of scores or real measurement data on one or more attributes but also include data over several replications (Kroonenberg, 1995).

Another complicating matter in determining the pattern of genotypic responses across environments is the differential genotypic expression across environments (GEI). If GEI exists, means across trials are of limited use for meaningful recommendation tasks (Voltas *et al*. 2005). According to Yan and Hunt (2001), understanding the causes of GEI is useful for establishing breeding objectives, identifying ideal test environments and to formulate recommendations with regard to optimal genotype adaptation to areas. A model to clearly distinguish between the main and interaction effects is thus needed.

Several statistical models have been proposed for increasing the chance of exploiting positive GEI and supporting breeding programme decisions in variety/cultivar selection and recommendation for target set of environments (DeLacy *et al*., 1996). Additive main effects and multiplicative interactions (AMMI) and site regression (SREG) genotype plus genotype-by-environment interaction (GGE) models are the current models that effectively capture the additive (linear) and multiplicative (bilinear) components of GEI and provide meaningful interpretation of multi-environment data set in breeding programs (Gauch and Zobel, 1988, 1997; Annicchiarico, 1997; Yan, 1999; Yan *et al*., 2000).

In Chapters 4 and 5, the AMMI model was discussed with regard to G, E and GEI amongst some citrus scion and rootstock combinations. The AMMI model analysis combines the additive (linear) parameters of traditional ANOVA with multiplicative (bilinear) parameters of principal component analysis (PCA) and according to Gauch (2006), as it has both linear and bilinear component of GEI, it is very useful in visualizing multi-environment data and gaining accuracy. AMMI provides a visual representation of patterns in the data through a biplot that makes use of the first interaction principal component axis (IPCA1) and the mean of the measured entities (e.g. yield) of both the genotype and the environments (Aina *et al*. 2007).

Gabriel (1971) was the first to formulate the concept of biplots which has become a popular data visualisation tool in many scientific research areas, including psychology, medicine, business, sociology, ecology, and agricultural sciences.

Mathematically, a biplot may be regarded as a graphical display of matrix multiplication (Yan and Tinker, 2006). The reasoning followed for biplots is that any two-way table or matrix X that contains m columns and n rows can be regarded as the product of two matrices namely A with n rows and B with m columns and r rows. Matrix X can thus be mathematically decomposed to its two components, A and B (Figure 6.1).

Matrix X is referred to as a rank-two matrix if the number of rows (r) is two. When r = 2, the two-way table X is said to be a rank-2 matrix and can be displayed in a 2-D biplot exactly (Yan and Tinker, 2005). Each row in Matrix A has two values, which define a point in a two-dimensional plot. Correspondingly, each column in Matrix B have two values, which also defines a point in two dimensional plot. When both the n rows of A and m columns of B are displayed in a single plot, this plot is called a biplot (Yan, 2001). Therefor the biplot of a rank-two matrix contains only n+m points compared to the nxm points of the original matrix but retain all of the original matrix information.

Greenacre (2010) illustrated the visual concept of a biplot in a very simple way through comparing a GEI table (Matrix X a 5x4 matrix) mathematically decomposed to its two components, A (5x2 matrix) and B (2x4 matrix).

Matirx X = Matrix A x Matrix B

$$\begin{bmatrix} & m1 & m2 & m3 & m4 \\ n1 & m1n1 & m2n1 & m3n1 & m4n1 \\ n2 & m1n2 & m2n2 & m3n2 & m4n2 \\ n3 & m1n3 & m2n3 & m3n3 & m4n3 \\ n4 & m1n4 & m2n4 & m3n4 & m4n4 \\ n5 & m1n5 & m2n5 & m3n5 & m4n5 \end{bmatrix} = \begin{bmatrix} c1n1 & c2n1 \\ c1n2 & c2n2 \\ c1n3 & c2n3 \\ c1n4 & c2n4 \\ c1n5 & c2n5 \end{bmatrix} \begin{bmatrix} m1r1 & m2r1 & m3r1 & m4r1 \\ m1r2 & m2r2 & m3r2 & m4r2 \end{bmatrix}$$

$$m1n1 = (c1n1 * m1r1) + (c2n1 * r2m1)$$

$$m4n5 = (c1n5 * m4r1) + (c2n5 * m4r2)$$

Figure 6.1 Decomposition of a Matrix X in to its two components, A and B

For illustration purposes, data has been added, for yield of five genotypes in four environments (Figure 6.2).

Matirx X = Matrix A x Matrix B

$$\begin{bmatrix} & m1 & m2 & m3 & m4 \\ n1 & 8 & 2 & 2 & -6 \\ n2 & 5 & 0 & 3 & -4 \\ n3 & -2 & -3 & 3 & 1 \\ n4 & 2 & 3 & -3 & -1 \\ n5 & 4 & 6 & -6 & -2 \end{bmatrix} = \begin{bmatrix} n1 & n2 \\ 2 & 2 \\ 1 & 2 \\ -1 & 1 \\ 1 & -1 \\ 2 & -2 \end{bmatrix} \begin{bmatrix} m1 & m2 & m3 & m4 \\ 3 & 2 & -1 & -2 \\ 1 & -1 & 2 & -1 \end{bmatrix}$$

$m1n1 = (2 * 3) + (2 * 1) = 8$

$m2n3 = (-1 * 2) + (1 * -1) = -3$

$m3n5 = (2 * -1) + (-2 * 2) = -6$

$m1n5 = (2 * 3) + (-2 * 1) = 4$

Figure 6.2 GEI table of five genotypes in four environments (Matrix X) (5x4 matrix) mathematically decomposed to its two components, A and B respectively 5x2 and 2x4 matrices

The "sum of cross products" $(e.g. m1n1)$ illustrated in Figures 6.1 and 6.2 is called the inner or scalar products of the row vectors (Y_{e1}, Y_{e2}) and column vectors (Z_{g1}, Z_{g2}) and forms the basis of the biplot geometry (Kroonenberg, 1995; Greenacre, 2010). A biplot is obtained by representing each row as a point Y_e with coordinates Y_{e1} and Y_{e2} and each column as point Z_g with coordinates Z_{g1} and Z_{g2} in a two dimensional graph (with origin=0). These points are generally referred to as column and row makers respectively (Kroonenberg, 1995).

After decomposition of the data into its two component matrices the five genotypes and four environments can be presented in a biplot like Figure 6.3. In Figure 6.3 one of the sets of points, in this case environments, are drawn as vectors connected to the origin. In graphs, genotypes are usually represented by points and environments by vectors. This choice is preferred because genotypes are normally compared with regard to their performance in an environment (Kroonenberg, 1995).

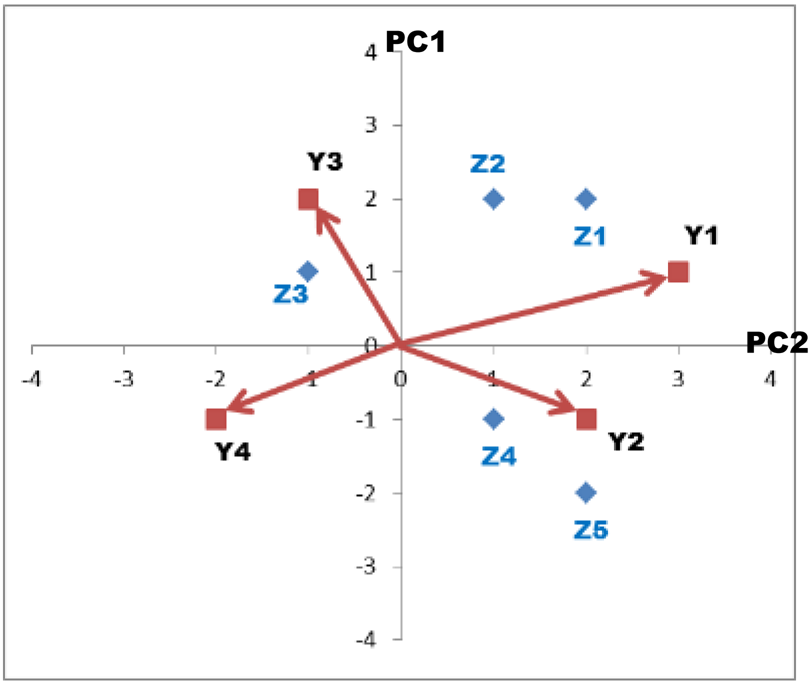

Figure 6.3 The geometry of biplot compiled from the GEI table of five genotypes in four environments (Matrix X a 5x4 matrix). Y1, Y2, Y3 and Y4 are four hypothetical environments and Z1, Z2, Z3, Z4 and Z5 are five hypothetical genotypes; PC1 and PC2 are first and second principle components respectively

To determine the relationship or interaction of two genotypes with the same environment, the lengths of their projections onto that environment can be compared. In Figure 6.3 each of the vectors connected to the origin defines a biplot axis onto which the genotypes can be projected (dropping the genotype point perpendicular onto the vector as in Figure 6.4.).

According to Yan (2001) the yield of genotype *i* in environment *j* (thus GEI for yield) is:

$$\boldsymbol{YLD_{ij} = \overline{OY_j} cos\theta_{ij} \overline{OZ_i} = \overline{OY_j}\, OP_{ij}} \quad \textbf{[E 1]}$$

- $\overline{OY_j}$ is the absolute distance from the biplot origin O to the marker of genotype *i*
- $\overline{OZ_i}$ is the absolute distance from the biplot origin O to the marker of the environment *j*
- θ_{ij} is the angle between the vectors OZ_i and OY_j
- $\overline{\boldsymbol{OP_{ij}}}$is the projection of the marker of genotype *i* to the vector of environment *j*

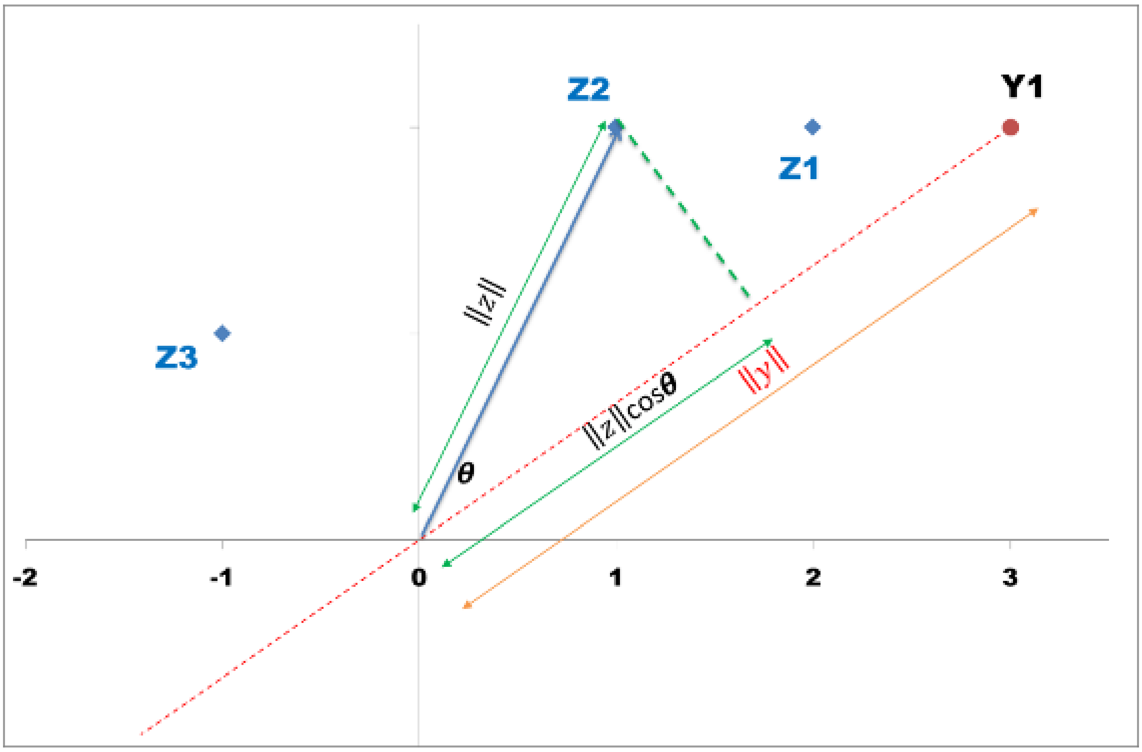

Figure 6.4 An example of two points Y1 and Z2 whose vectors subtend an angle of *θ* with respect to the origin

Interpreting the biplot (Kroonenberg, 1995):

- The relationship between a genotype vector (OZ_j) and an environment vector (OY_i) is positive if their angle is acute and negative if the angle is obtuse.
- When the projection of a marker Z_i onto the environment vector OY_i coincides with the origin then GEI for yield (YLD_{ij}) equals zero.
- A positive value for YLD_{ij} indicates that the genotype *i* has a high score in that environment (*j*) relative to the average score in that environment.

A biplot is obviously a display of an ideal rank-two matrix. In reality, however, a two data set is rarely exactly a rank-two matrix. According to Yan (2001) the process described to decompose Matrix X into component matrices A and B is called singular value decomposition (SVD). SVD results in r principle components (PCs). The number of PCs will be equal to the smaller of the n or m (Kroonenberg, 1995; Yan, 2001). However, by approximation of Matrix X with a rank two matrix (Figure 6.2), only the first two principle components (PCA1 and PC2) are used to present the original matrix X. Thus if PC 1 and PC 2 explain a large portion of the total variance of X then X is said to be sufficiently approximated by a rank-two matrix and can be approximately displayed in a biplot (Yan, 2001).

A genotype's response to different environments is multivariate (Lin *et al*., 1986). According to Kandus *et al*. (2010) the purpose of multivarate analysis is threefold:

a. to eliminate "noise" in the data
b. to summarise the information and
c. to reveal structure in the data and improve the accuracy of yield estimates

AMMI biplot: By using a number of axes such as genotype and environment means as well as individual values for principal components the AMMI analysis converts the structure of the data, which are originally in the form of matrices, into a smaller scale (Gauch and Zobel, 1988, Kahram, 2013). AMMI presents the results graphically in an easily interpretable biplot and can identify high yielding, widely adapted genotypes that are superior over sites of interest and, if this is not possible, specific varieties for specific areas.

According to Gauch (1992) AMMI is the only model that can clearly distinguish between the main and interaction effects. The AMMI produces reliable estimates of genotype and environment performance and summarizes the relationships between these components graphically into biplots. According to Crossa (1990) the AMMI model is equal to an increase of replicates from two to five.

Commonly used AMMI biplots are the AMMI 1 and AMMI 2 biplots. Of these the AMMI1, which plots the interaction principal component (IPCA1) scores against the genotype and environment means is the most used. The genotype and environment main effects, the stability of genotypes, the relative adaptability of genotypes to different environments, and the mega-environments represented by the data are visualised by this biplot. An alternative method for determining if genotypes have the same relative performance in different environments is by directly computing stability from the genotype's contribution to the interaction sum of square and is called the AMMI Stability Value or ASV (Purchase and Hatting, 2000).

For a better understanding of the results and the discussion the following points regarding biplots must be taken into account:

- The centre of the AMMI 1 biplot shows the mean of the genotypes/environments on the X-axis and the mean of the genotypes/environment scores on the y-axis (Kempton, 1984; Kroonenberg, 1995).
- Genotypes grouped together tend to have similar yield responses and similar patterns of responses over environments (Smith, 1992)
- Genotypes and environments with scores close to zero (y-axis) are deemed to have a small interaction as explained in Figure 6.4 (Kroonenberg, 1995; Rashidi *et al*., 2013).

GGE biplot: GGE is an acronym for genotype main effect (G) plus GEI, which is the only source of variation that is relevant to cultivar evaluation. According to Gauch and Zobel (1996) it is a usual phenomenon that the environment is the predominant source of yield variation in most multi-environment trials, while the contribution of G and GEI are relatively small. In genotype evaluation, only this relatively small contribution of G and GEI is relevant, particularly when GEI is identified as repeatable (Hammer and Cooper, 1996) therefore Yan *et al*. (2000) combined the two terms and referred to it as GGE.

Mathematically, GGE is the G by E data matrix after the environment means are subtracted and the GGE biplot is consequently the visualisation tool, which graphically displays a GEI in a two-way table (Yan *et al.*, 2000). In this regard the GGE biplot method also makes use of the same mathematical principals as AMMI but differs based on how the two-way table of G by E means are treated before performing SVD. The GGE biplot method applies SVD to the data minus the environment means only while AMMI applies SVD to the data minus the genotype and environment means (Gauch, 2006). Conventional AMMI biplots therefore describe only GEI effects, while GGE biplots describe genotype and GEI effects.

According to Yan and Kang (2003) analysis of GEI data should identify mega-environments, identify the best (most discriminative but also representative) test environment and also indicate the performance and adaptability of genotypes.

A GGE biplot is an effective tool to visualise the data in a two-way table from three perspectives:

i. the interrelationship amongst entries and testers or mega-environment analysis (e.g. “which-won-where” pattern), whereby specific genotypes can be recommended to specific **mega-environments** (Yan and Kang, 2003; Yan and Tinker, 2006)
ii. the interrelationship among the entries thus **genotype evaluation** (the mean performance and stability), and
iii. the interrelationship among testers thus **environmental evaluation** (the power to discriminate among genotypes in target environments).

Comment 1: *Entries and testers are the two factors in a two-way data table. Entry-factor is the factor to be tested and the tester-factor is the factor used to test it. An entry is a level of the entry factor while the tester is the level of the tester factor. In GE data genotypes are entries and environments are testers. In genotype by trait data genotypes are still entries and the traits are the testers. The convention in statistics is to present entries as rows and testers as columns in a data matrix.*

Mega environment analysis: Yan and Rajcan (2002) defined a mega-environment as a group of localities that consistently share the same best cultivar(s), while Gauch and Zobel (1996) defined a mega-environment as a portion of a crops’ growing region with a homogenous environment that causes some genotypes to perform similarly. The mega environment comparison view of the GGE biplot or commonly known as a “which-won-where” pattern (Yan *et al*., 2000) or in short the polygon view is an effective visual tool in mega-environment analysis (Yan *et al*., 2007). It not only addresses mega-environment differentiation but also other important issues such as crossover GEI and specific adaptation (Yan and Tinker, 2006). The mega-environment analysis is done through the construction of a convex hull (Yan, 1999) and pairwise comparison (Yan and Tinker, 2006).

Construction of a convex hull and pairwise comparison: In mathematics, the convex hull or convex envelope of a set S of points in the Euclidean plane or Euclidean space is the smallest convex set that contains S. For instance, when S is a bounded subset of the plane, the convex hull may be visualised as the shape formed by a rubber band stretched around S. The convex hull of S is thus the smallest convex polygon that contains all the points of S (de Berg *et al*., 2000).

Following the rules of a convex hull an irregular polygon is formed by connecting the genotype markers that are the furthest from the biplot origin in such a way that the rest of the genotypes are contained in the polygon (Kaya, 2006). Based on pairwise comparison of two genotypes, the vertices of the hull are then bisected by perpendicular lines through the origin. Pairwise comparison of genotypes state that two genotypes can visually be compared by connecting them with a straight line and then using a perpendicular line, connect this line with the origin. This is called the equality line of the two genotypes and the equality lines divide the biplot into sectors with the winning genotype located at the "corner" thus defining a "mega-environment" (Yan, 1999; Kaya *et al.* 2006; Yan and Tinker, 2006).

If all environment markers in a polygon view fall into a single sector of the convex hull, it means that, to a rank-two approximation, a single cultivar had the highest yield in all environments. (a rank-two approximation is a matrix resulting from multiplying a matrix with two columns by a matrix with two rows). If environment markers fall into different sectors, it constitutes a crossover GEI pattern as it indicates that different cultivars won in different sectors. If this crossover GEI pattern is not repeatable across years, the GEI cannot be exploited. It should therefore rather be avoided through the selection of genotypes with high but stable yields across the target environments (Yan *et al*., 2007).

Genotype evaluation: According to Yan *et al*. (2007) only after the mega-environment issue is addressed does genotype evaluation and test-environment evaluation become meaningful. Yield performance and stability of genotypes can be evaluated by an average environment coordination method (AECM) (Yan, 2001; Yan and Hunt, 2002; Yan, 2002). Test environments and its representativeness and discriminating value can also be evaluated by an AECM (Yan, 2001; Yan and Tinker, 2006).

The AEC method for interrelationship determination: The specific environment (or genotype) is viewed as an "ideal" environment (or genotype), and concentric circles are plotted around it. The closer an environment (or genotype) is to the "ideal" environment (or genotype) the more attributes they share. In this method, an average environment is defined by an "average environment coordinate" (AEC) which is the average PC1 and PC2 scores of all environments and is represented by a small circle on the biplot (Blanche, 2005, Blanche and Meyers, 2006).

- A line is then drawn to pass through the AEC and the biplot origin; this line is called the average environment axis and serves as the abscissa of the AEC. The AEA is a measure of the representativeness of the average environment (Tukamuhabwa *et al*., 2012). The AEC abscissa has one direction, with the arrow pointing to greater genotype main effect (Kaya *et al.*, 2006).
- The ordinate of the AEC is the line that passes through the origin and is perpendicular to the AEC abscissa. Unlike the AEC abscissa, which has one direction, the AEC ordinate has two arrows each facing away from the biplot origin, pointing towards greater GEI effect, and reduced stability. However, in the Genstat 15th edition only the AEA has an arrow while the two arrows on the AEC ordinate is omitted (Blanche, 2005; Blanche and Meyers, 2006).
- The AEC ordinate separates genotypes with below-average means from those with above-average means. Furthermore, the average yield of genotypes is approximated by the projections of their markers to the AEC abscissa.

The mean vs. stability coordination view of the GGE biplot: is produced using the the AEC method as described, to facilitate genotype comparisons based on mean performance and stability across environments within a mega-environment (Blanche and Meyers, 2006, Yan *et al.,* 2007). The projections of the genotype markers on the AEA are proportional to the rank-two approximation of the genotype means due to the inner-product property of the biplot and thus represent the main effects of the genotypes, G. The arrow of the AEA thus points in the direction of higher mean performance of the genotypes, thereby automatically ranking the genotypes with respect to mean performance. An increased vertical distance from the AEA parallel to the AEC ordinate indicates a greater tendency for GE interactions of the genotype and therefore a tendency to be more variable and less stable across environments. Likewise, genotypes that cluster together on the AEA, with a short projection on the AEA, is highly stable and would thus perform consistently across environments.

Projections can also be done on the AEC ordinate or stability line and in this case a longer projection of a genotype onto the stability line would indicates a greater tendency for GE interactions and thus instability.

Test-environment evaluation: An "ideal" test environment should be both discriminating of the genotypes and representative of the mega-environment. Test-environment evaluation in a GGE analysis is by means of an environment comparison view. This view is based on an environment-centred G by E table without any scaling (scaling =0) and is environment-metric preserved (SVP=2) in other words the singular values were entirely partitioned into the environment scores.

The vector length of an environment represents its discriminating ability: the longer the vector, the more discriminating the environment, while the projection of the vector length of an environment onto the AEC ordinate is a measure of its representativeness: the longer the projection, the less representative the environment.

The evaluation of tree fruit genotypes is an expensive and protracted exercise due to the area needed per genotype and an approximate eight years from planting to stable yielding mature trees. There are thus two issues to keep in mind when evaluating fruit trees. First of all the evaluation or "ideal" test environment should be able to discriminate amongst the large number of genotypes from the breeding programme in order to find the best genotypes. However, the test environments within a MET should also be representative of the mega-environments within the growing region of the fruit crop in order to recommend the best genotype or predict a genotype's performance on a specific producer's land. Determining mega environments and suitable test environments can prevent duplication and/or exclusion with regard to trial environments thus resulting in an evaluation programme with enhanced efficiency.

The objective of this chapter was to demonstrate the illustrative value of AMMI and GGE biplots with regard to accurate and fast data interpretation.

6.2 MATERIAL AND METHODS

6.2.1 Materials

6.2.1.1 Localities, scion and rootstock material

Data were taken from the same trial sites and years as discussed in Chapter 4. The propagation methods, orchard practices, trial layout, scions and rootstock genotypes were thus the same. For the purpose of illustration of the AMMI and GGE biplots the same data for mega environment identification and the same subset of data for genotype and test environment evaluation will be used.

6.2.2 Methods

6.2.2.1 Evaluation of traits

Yield data was taken according to the methods described in Chapter 4.

6.2.2.2 Statistical analysis and biplot generation

AMMI and GGE biplots were constructed using GenStat 15th Edition (Payne *et al*., 2012). GEI for fruit production was analysed according to a classical multiplicative model or AMMI (Gauch, 1992) with two multiplicative terms as discussed in Chapter 4 and GGE based on the model for two Principal Components according to Yan and Kang (2003). The data was subjected to:

i. an ANOVA to determine the presence of GEI in the data followed by,
ii. visualisation by means of AMMI and GGE biplot to define mega environments for rootstock evaluation,
iii. evaluation of genotypes and test environments within a mega environment with GGE biplots.

6.3 RESULTS AND DISCUSSION

6.3.1 AMMI biplot analysis

ANOVA: The AMMI ANOVA for yield of four rootstocks genotypes evaluated with five different citrus scion types (Figure 4.2) is presented in Table 6.1 and showed significant effects for genotype, environment and GEI with regard to yield for all five citrus scion types (trials).

Table 6.1 AMMI analysis of variance for yield of four rootstocks genotypes (G) evaluated with five different citrus scion types (E)

Source of variance	df	SS	MS	Probability (P≤0.05)	% Variance explained: Treatment Variance	% Variance explained: AMMI 1 biplot	% Variance explained: Interaction components
Total	79	118064	1494				
Treatment	19	115052	6055	0.00			
E (Scion types)	4	97759	24440	0.00	84.97	**84.97**	
Block	15	893	60	0.26			
G (Rootstocks)	3	9690	3230	0.00	8.42	**8.42**	
Interaction	**12**	**7602**	**634**	**0.00**	6.61		
IPCA 1	**6**	**5598**	**933**	**0.00**		**4.87**	**73.64**
IPCA 2	**4**	**1721**	**430**	**0.00**			**22.64**
Residual	2	283	142	0.06			3.72
Error	45	2119	47				
					100.00	98.23	100.00

The model revealed that differences between the environments (scions) accounted for 84.97% of the treatment SS. Genotypes (rootstocks) and GEI also accounted significantly for 8.42 and 6.61% of variation respectively. The SS for the first principal component axis (IPCA) was significant at P<0.05 and captured 73.64% of the interaction SS in 50% of the df. The other 26.36% of the variation (within 50% of the interaction df) was left in the residual.

The mean yields of the four genotypes grown in five environments (citrus scion types), the environment means and the IPCA 1 and IPCA 2 scores are presented in Table 6.2.

Table 6.2 Mean yield of four rootstock genotypes (G) budded with five Citrus scion types (E) with the first and second interaction principal component of genotype and environment

Rootstocks (G)		Genotype no.	G mean yield (kg.ha^{-1})	IPCA 1	IPCA 2
306		G1	55.93	1.59	3.66
42		G2	68.43	3.98	-1.97
575		G3	86.10	-4.12	0.17
608		G4	64.39	-1.45	-1.86
Citrus scion types	**(E)**	Environment no.	E mean yield (kg.ha^{-1})	IPCA 1	IPCA 2
Ellendale	(Ell)	E1	55.76	1.24	1.46
Grapefruit	(Grf)	E2	130.33	-4.12	-1.06
Mandarin	(Man)	E3	38.06	3.52	0.95
Midseason	(Mid)	E4	36.87	1.46	-3.47
Valencia	(Val)	E5	82.54	-2.10	2.13

AMMI 1: A biplot of the AMMI 1 results (Table 6.1) with the x-axis showings the main effects (rootstock and environment mean yields) and the y-axis showing the first IPCA scores is presented in Figure 6.5. The biplot accounted for 98.23% of the treatment SS. This is calculated by the percentage variance explained by the treatment SS for G, E and IPCA1 (see Table 6.1). The mean environment yields (citrus scion types) ranged from 36.87 kg ha-1 for midseason to 130.33 kg ha^{-1} for grapefruit. The environments (citrus scion types) showed much variability in both main effects and interactions (Table 6.2).

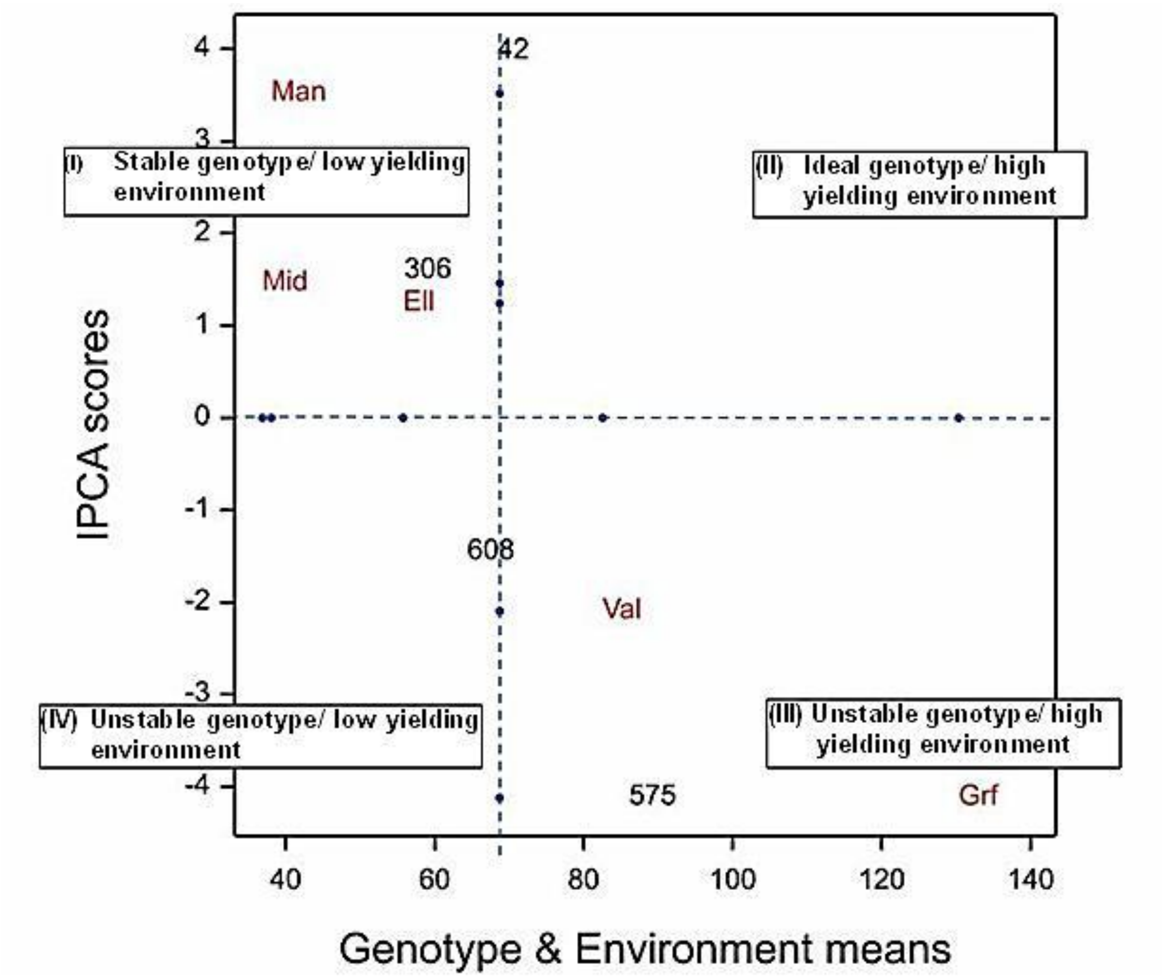

Figure 6.5 AMMI model 1 biplot for yield of four rootstocks genotypes (G) evaluated with five different citrus scion types (E) with main effects as the abscissa and PC1 for its ordinate accounting for 98.23% of the treatment SS.

The comprehensive pattern of the data set can be visualised in this biplot and it is possible to see, through closer inspection, whether the rootstocks (genotypes) or citrus scion types (environments) differ in their main effects, interactions, or both.

According to Crossa *et al*. (1990) genotypes or environments that appear almost on a perpendicular line of the graph in the biplot display, had similar mean yields and those that fall almost on a horizontal line had similar interaction. From the biplot it is thus evident that rootstocks 42 and 608 have similar mean yields and none had similar interaction. There were no high potential environments (citrus scion types) in quadrant II or low potential environments (citrus scion types) in quadrant IV. On the biplot the points for the generally adapted rootstock genotypes would be on the right hand side of the grand mean thus high performance and close to the horizontal (IPCA=0) line thus negligible GEI. Genotype 306 and 42 revealed a specific adaptability for citrus scion type midseason, Ellendale and mandarins, while 575 was specifically adapted to Valencia and grapefruit. Long vector lengths, from the centre of the biplot indicate that rootstock genotypes 575 and 42 had a large interaction with the environment (citrus scion types) in this case Valencia and grapefruit for 575 and mandarin for 42. A pattern could be observed from AMMI 1 indicating that there were mega-environments involved.

AMMI 2: The IPCA1 versus IPCA2 biplot (i.e. AMMI 2 biplot) explain the magnitude of interaction of each genotype and environment (Gauch and Zobel, 1988). An AMMI 2 biplot (Figure 6.6) was constructed of the IPCA 1 scores versus the IPCA 2 scores of the rootstock and environment (citrus scion types) mean yields.

In Table 6.1 GEI contributed 6.61% to the treatment SS. IPCA 1 accounted for 73.64% of the interaction SS and IPCA 2 accounted for 22.64%. The genotypes and environments that are the furthest away from the centre (origin) of the graph are the most responsive or interactive with the most GEI. Rootstock genotypes and environments (citrus scion ypes) that fall in the same sector interact positively and the genotypes are therefore best suited to that environment while those in opposite sectors interact negatively.

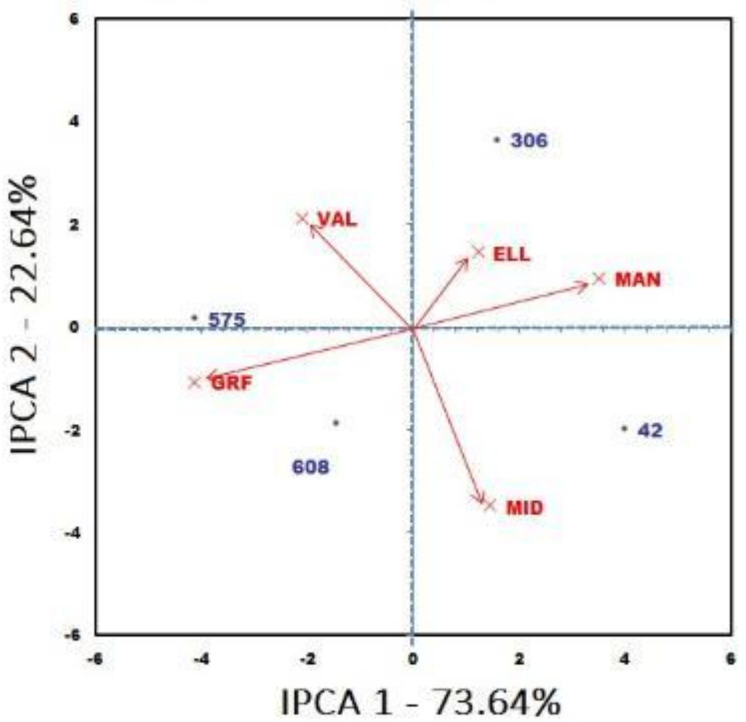

Figure 6.6 AMMI 2 biplot of the IPCA1 versus the IPCA2 for yield of four rootstocks genotypes (G) evaluated with five different citrus scion types (E)

In Figure 6.6 all the rootstock genotypes scattered away from the origin with 575 and 42 the furthest away indicating that they were more susceptible to the interactive forces of the environment. Interaction of rootstock genotypes with specific environments (citrus scion types) was determined by the projection of the rootstock genotype on the environment (citrus scion types) as per the method in Figure 6.4. Acute angles signified positive interaction such as genotype 575 with Grf and Val, 306 with Ell and 42 with Man.

AMMI polygon: When the extreme rootstock genotypes on a GE biplot (Figure 6.7) were connected to form a polygon (convex hull method) and perpendicular lines drawn from the centre of the biplots to meet the sides of the polygon, four sectors were recognised. This view revealed three mega environments: sector I with grapefruit and Valencia associated, sector II with Ellendale and mandarin and midseason in sector III. With the present data set, four sectors of which three had an environment (citrus scion types) were recognised with G3, G1, G2 and G4 at the vertex of the quadrilateral signifying a cross over interaction. Ell with the shortest vector was non-informative, as it provides little information on the genotypes and, therefore, should not be used as test environment (citrus scion types).

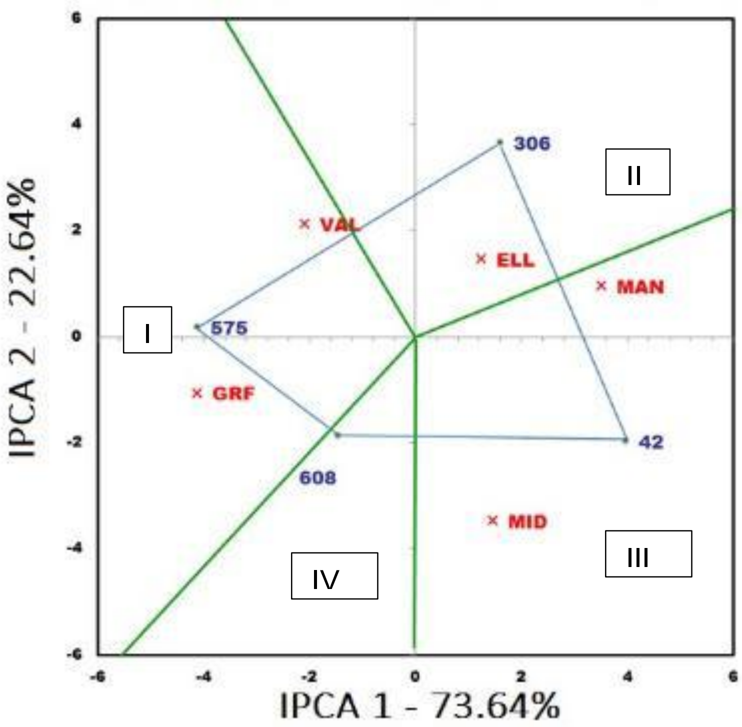

Figure 6.7 AMMI polygon view based on the AMMI 2 biplot

In Table 6.3 AMMI stability values (ASV), and ranking orders of the four rootstock genotypes tested across five environments (citrus scion types) are depicted. In the ASV method, a genotype with the lowest ASV score is the most stable, accordingly genotype G4 (608), followed by G1 (306) were the most stable, while genotypes G2 (42) and G3 (575) were unstable.

Table 6.3 AMMI stability values (ASV), and ranking orders of the four rootstock genotypes tested across five environments (citrus scion types).

Code	Genotype	Yield		IPCA 1	IPCA 2	ASV	
		Mean	Rank			Mean	Rank
G3	575	86.10	1	-4.12	0.17	13.39	4
G2	42	68.43	2	3.98	-1.97	13.08	3
G4	608	64.39	3	-1.45	-1.86	5.08	1
G1	306	55.93	4	1.59	3.66	6.35	2

Biplot analysis and ordination techniques revealed high significant differences for IPCA1 and IPCA2. Thus, based on AMMI 1 and 2 model and ASV ranking, G3 (575) was identified to be the best yielding but most unstable rootstock genotype while G4 (608) was the most stable rootstock genotype but ranked third with regard to yield potential.

Mega-environment is followed by genotype evaluation and test-environment evaluation within a mega environment (Yan *et al*., 2007).

Rootstock genotype evaluation within a mega environment (a specific citrus scion type): Mega environment determination grouped grapefruit and Valencia together as a high yielding mega environment (citrus scion type). Likewise midseason and mandarin were classified as a single mega environment while Ellendale, midseason and mandarins were all low yielding environments (citrus scion type).

Following an annual Duncan's multiple range test (MRT) at fixed significance level of 5% (P=0.05) for the Valencia group at Malalane it was found in Chapter 3 that all of the new cultivars were better than the standard 1043a wiith no significant difference amongst them and could be recommended and that the best rootstock for yield was 'Volckameriana'-575. The AMMI 1 analysis (Table 6.4) and biplot in Figure 6.8 concurs with this data, showing all of the Valencia selection except 1043b and 1044 to have yields higher than the average.

Table 6.4 AMMI analysis of variance for yield of four rootstocks (G) genotypes evaluated with eight scion selections (E) within the Valencia group (mega-environment)

					% Variance explained		
Source	df	SS	MS	Probability ($P \leq 0.05$)	TRT Variance	AMMI 1 biplot	Interaction components
Total	127	126441	996				
Treatment	31	97027	3130	0.00			
Genotype (G)	3	38345	12782	0.00	39.52	39.52	
Block	12	3795	316	0.42			
Environment (E)	7	43204	6172	0.00	44.53	44.53	
Interaction	**21**	**15478**	**737**	**0.00**	15.95		
IPCA	**9**	**8860**	**984**	**0.00**		9.13	57.24
IPCA	**7**	**3795**	**542**	**0.10**			24.52
Residual	5	2823	565	0.11			18.24
Error	84	25619	305				
					100.00	93.18	100.00

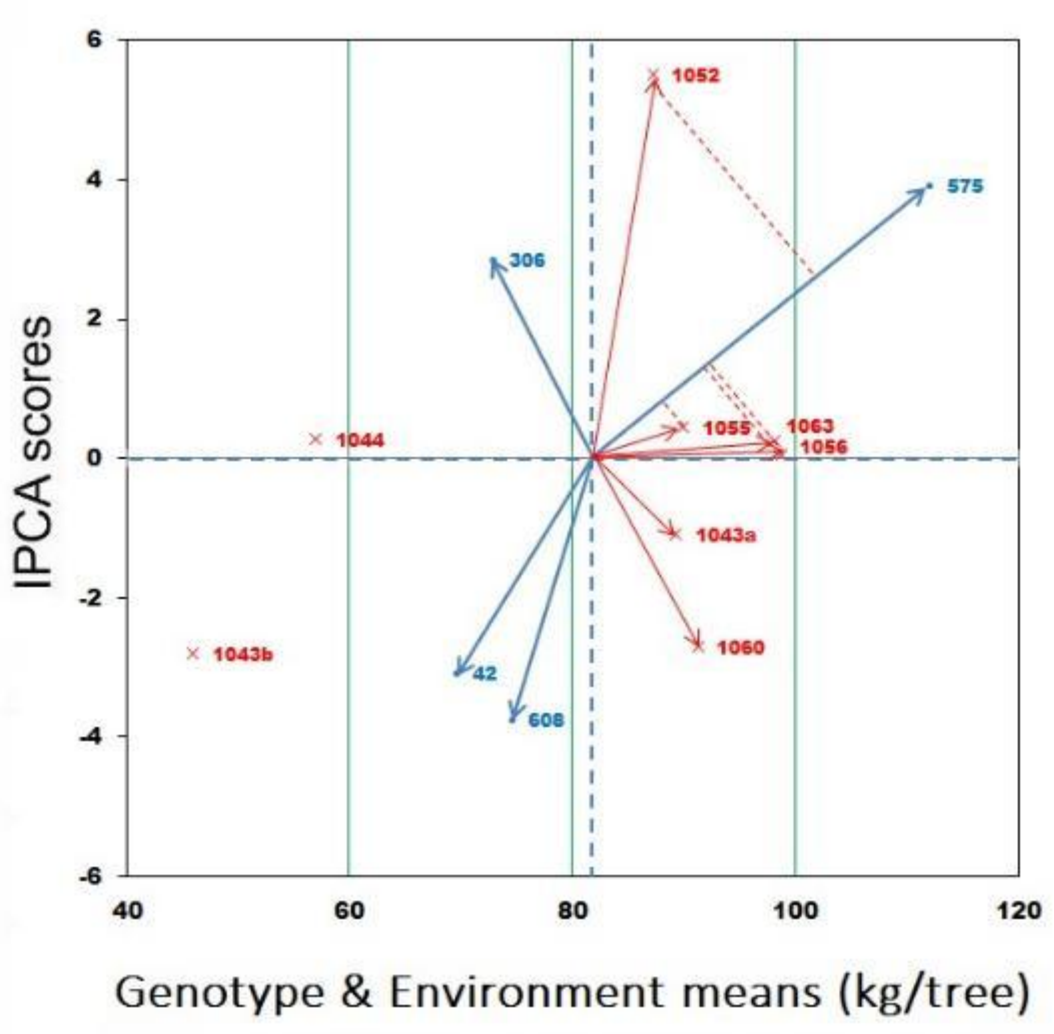

Figure 6.8 AMMI 1 biplot of the rootstock by scion interactions of eight Valencia selections and four rootstock genotypes showing the genotype and environment scores versus the mean

Figure 6.8 represents an AMMI 1 biplot of the rootstock (G) by scion (E) interactions of eight Valencia and four rootstock genotypes with the x-axis showings the main effects (rootstock and environment mean yields) and the y-axis showing the first IPCA scores. The AMMI 1 biplot (Figure 6.8) accounted for 93.18% (Table 6.4) of the treatment SS leaving 6.82% in the residual. However, it also represents the scion (G) by rootstock (E) interactions as shown in Table 6.4.

General adaptability of genotypes to all environments are, according to the AMMI model, characterised by means greater than the grand mean and IPCA scores close to zero (Rashidi *et al*. 2013). Genotypes that are characterised by high mean performance and large values for IPCA 1 scores are considered as having **specific adaptability to** the associated environments**.**

Table 6.4 indicated a significant interaction amongst rootstocks and scions (15.95%). With regard to rootstock genotypes (Figure 6.8) rootstock 575 was the only rootstock genotype that was characterised by means greater than the grand mean but as it had large values for IPCA 1, it was considered as having **specific adaptability to** the associated environments (Valencia selections): 1052, 1055, 1063 and 1056 (acute angles with rootstock 575). Contrary to these, the high yielding environments 1043a and 1060 exhibited negative interaction with rootstock 575 as indicated by the obtuse angle of the vectors with that of rootstock 575.

Rootstocks as environments produced the same percentages as in Table 6.4 but the genotype and environment interchanged (Table 4.12). Figure 6.8 can thus be used to identify the best scion genotypes as well. The AMMI biplot (Figure 6.8) revealed rootstock 575 as a high yielding environment and 42, 306 and 608 were all low yielding environments (rootstocks). Six of the eight scions had yields above the grand mean. According to Crossa *et al*. (1990) genotypes, Valencia selections 1052, 1055, 1043a and 1060 had similar mean yields as they appear almost on a perpendicular line of the graph in the biplot while 1056, 1063 and 1055 falling almost on a horizontal line had similar interaction.

Regarding rootstock by scion interaction, the AMMI biplot indicated that Valencia selection 1052 had a large GEI, while Valencia selection 1055 would be the best performing scion if grafted to rootstocks 575 and Valencia selections 1056 and 1063 was the more generally adapted genotypes. AMMI tables for the yield of scion (G) and rootstocks (G) genotypes evaluated for five consecutive years (E) at one locality with eight selections (E) within the Valencia group are presented respectively in Table 6.5 and Table 6.6 and the biplots in Figure 6.9 and Figure 6.10.

The IPCA 2 values were determined to be non-significant ($P \leq 0.05$) for scion by rootstock, rootstock by scion and scion by year effects and consequently no AMMI 2 biplots were drafted.

Table 6.5 AMMI analysis of variance for yield of scion (G) genotypes evaluated for five consecutive years (E) at one locality with eight selections (E) within the Valencia group

Source	df	SS	MS	Probability (P≤0.05)	% Variance explained TRT Variance	 **AMMI 1 biplot**	 Interaction components
Total	159	215402	1355				
Treatment	39	196764	5045	0.00			
Environment (years)	4	131315	3282 9	0.00	66.74	66.74	
Block	15	3265	218	0.12			
Genotype (Val selections)	7	54005	7715	0.00	27.45	27.45	
Interaction	**28**	**11443**	**409**	**0.00**	5.82		
IPCA	**10**	**7679**	**768**	**0.00**		3.90	67.11
IPCA	**8**	**2225**	**278**	**0.07**			19.44
Residual	10	1539	154	0.41			13.45
Error	105	15373	146				
					100.00	98.09	100.00

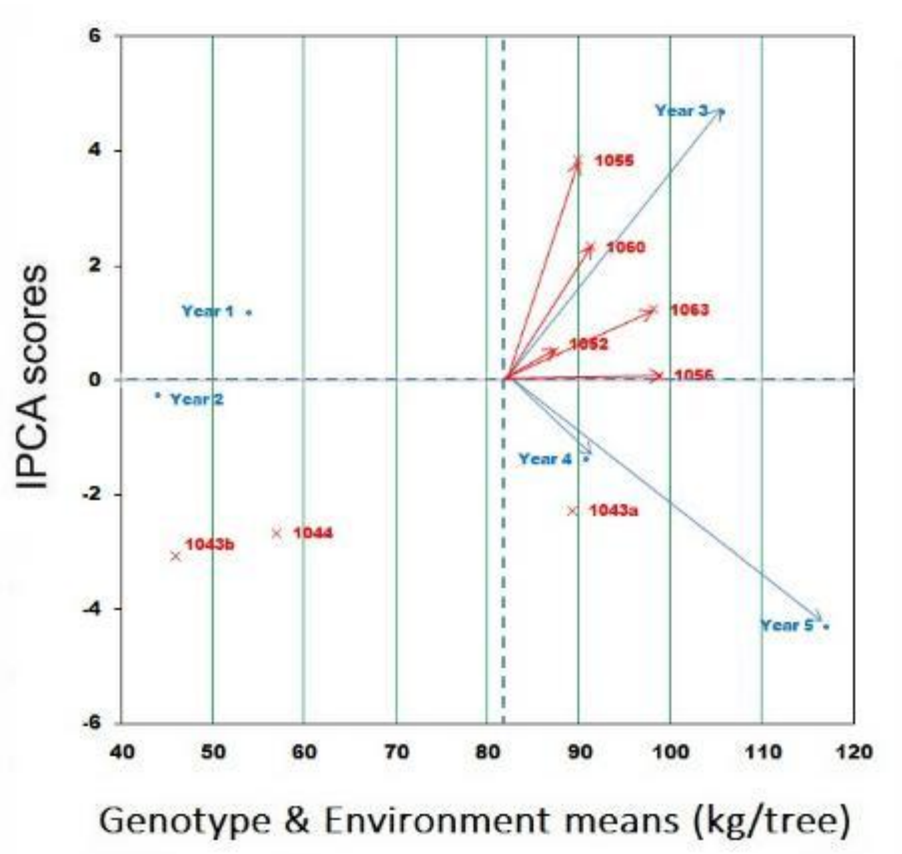

Figure 6.9 AMMI 1 biplot of the scion by year interactions of eight Valencia selections and four rootstock genotypes showing the genotype and environment scores versus the mean

Table 6.6 AMMI analysis of variance for yield of rootstock (G) genotypes evaluated for five consecutive years (E) at one locality with eight selections (E) within the Valencia group

					% Variance explained		
Source	df	SS	MS	Probability (P≤0.05)	TRT Variance	**AMMI 1 biplot**	Interaction components
Total	79	108328	1371				
Treatment	19	103221	5433	0.00			
Environment (Years)	4	65657	16414	0.00	63.61	**63.61**	
Block	15	1632	109	0.18			
Genotype (Rootstocks)	3	23965	7988	0.00	23.22	**23.22**	
Interaction	**12**	**13598**	**1133**	**0.00**	13.17		
IPCA	**6**	**11638**	**1940**	**0.00**		**11.27**	85.59
IPCA	**4**	**1928**	**482**	**0.00**			14.18
Residual	2	33	16	0.81			0.24
Error	45	3475	77				
					100.00	**98.10**	100.01

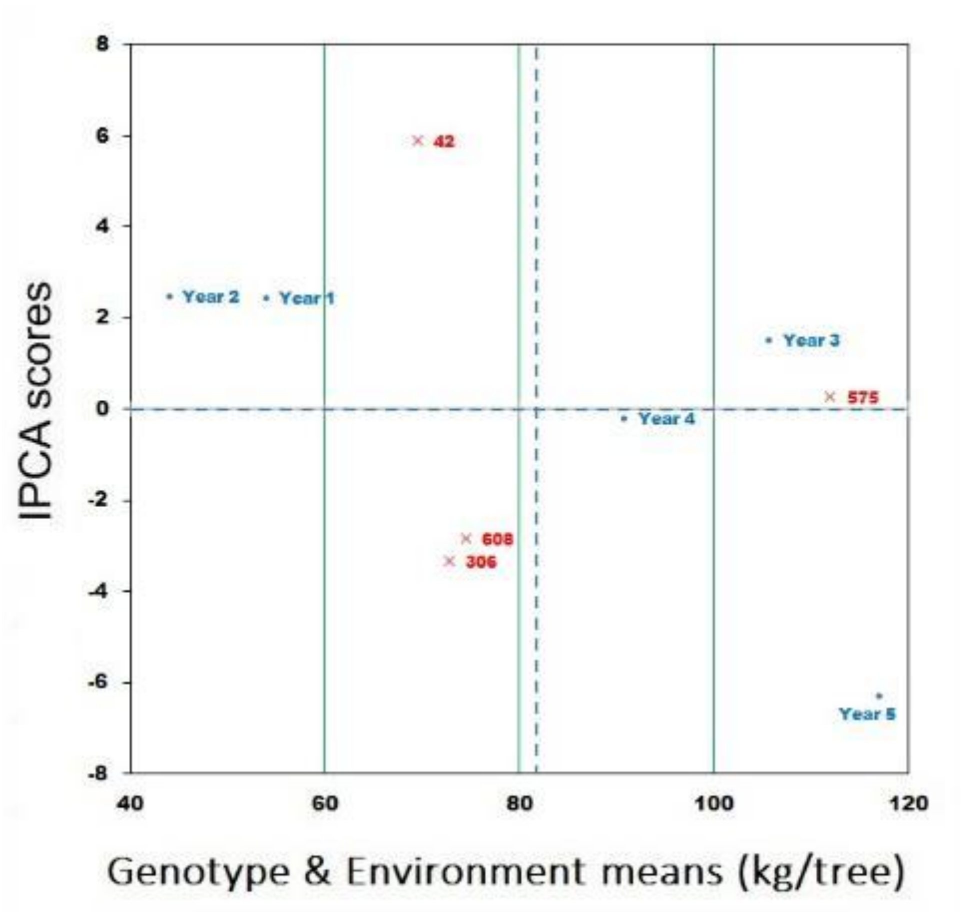

Figure 6.10 AMMI 1 biplot of the rootstock by year interactions of eight Valencia selections and four rootstock genotypes showing the genotype and environment scores versus the mean

An AMMI 1 biplot of the scion by year interactions of eight Valencia selections and four rootstock genotypes (Figure 6.9) accounted for 98.09% of the variation and confirmed Valencia scion genotypes 1043a, 1043b and 1044 to be low yielding genotypes across years.

Val scion genotype 1056 with a mean score larger than the grand mean and the IPCA scores close to zero can be considered as generally adapted to all years while Val genotypes 1060, 1052 and 1063 were specifically adapted to year three. All the years had long vector lengths indicating a large interaction with the genotypes associated with them.

The AMMI 1 biplot of the rootstock by year interactions of eight Valencia selections and four rootstock genotypes (Figure 6.10) accounted for 98.10% of the variation and confirmed rootstock genotypes 42, 306 and 608 to be low yielding rootstock genotypes across years. Rootstock 575 had a mean score larger than the grand mean and the IPCA scores almost equal to zero thus generally adapted to all years. Year one and two had similar GEI but different mean yields while year four was the most stable year exhibiting very little GEI.

6.3.2 GGE biplots analysis

According to Yan *et al*. (2007) the AMMI1 graph was designed to address the which-won-where pattern, while the GGE biplot is specifically used for mega-environment analysis based on genetic correlation between environment and the which-won-where pattern; test environment evaluation based on their discriminating ability and representativeness; and genotype evaluation based on their mean performance and stability across a mega-environment.

6.3.2.1 Mega environment analysis

Figure 6.11 represents the two scenarios of scions and rootstocks as explained in Figure 4.3 and Figure 4.4. In Fig 6.11(a), citrus scion types were deemed the environments and in Fig 6.11(b), the citrus scion types were deemed the environments. According to Yan (1999) mega environments are test environments with different winning genotypes located at the vertex of the polygon.

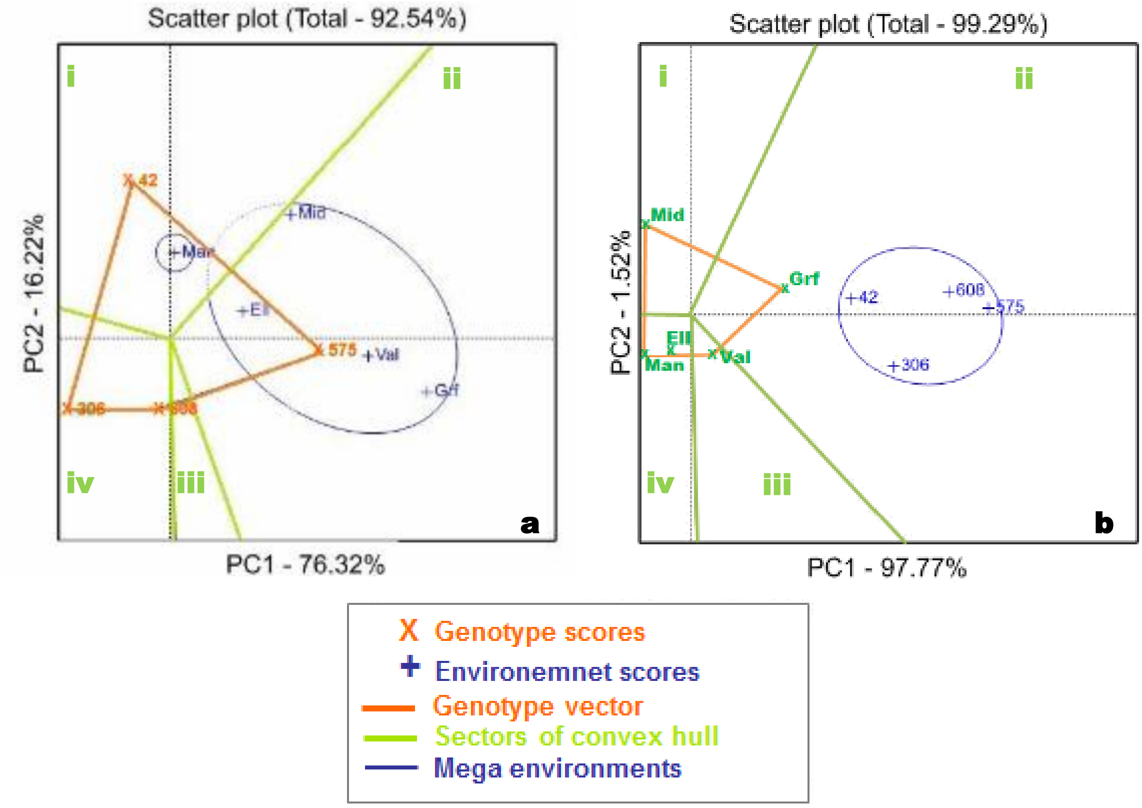

Figure 6.11 Polygon (which-won-where) view of the GGE biplot to show:(a) which rootstock genotypes performed best within five different citrus scion types over five years and (b) which citrus scion types performed best in association with what rootstocks

Based on the five environments (citrus scion types) (Figure 6.11a) used in the rootstocks study, four sectors and two mega environments with different "winning" genotypes were identified using a scatter plot with polygon bisectors. This biplot explained 92.54% of the variation. The biplot regarding the rootstocks as environments explained 99.29% of the variation observed (Figure 6.11b) and revealed four sectors but only one mega environment containing all off the rootstocks used.

6.3.2.2 Mega environment analysis for rootstock evaluation

Following pairwise comparison of two genotypes, the perpendicular that bisects the vertices of the convex hull (polygon) does not necessarily intersect the line between the two genotypes that it connects but rather the invisible extension thereof. This is evident in Figure 6.12 where the line connecting genotypes 306 and 608 on the vertices of the convex hulls is not intersected by the perpendicular but the invisible extension thereof (portrayed by a broken line for illustration) is, causing 306 and 608 to fall in the same sector namely v.

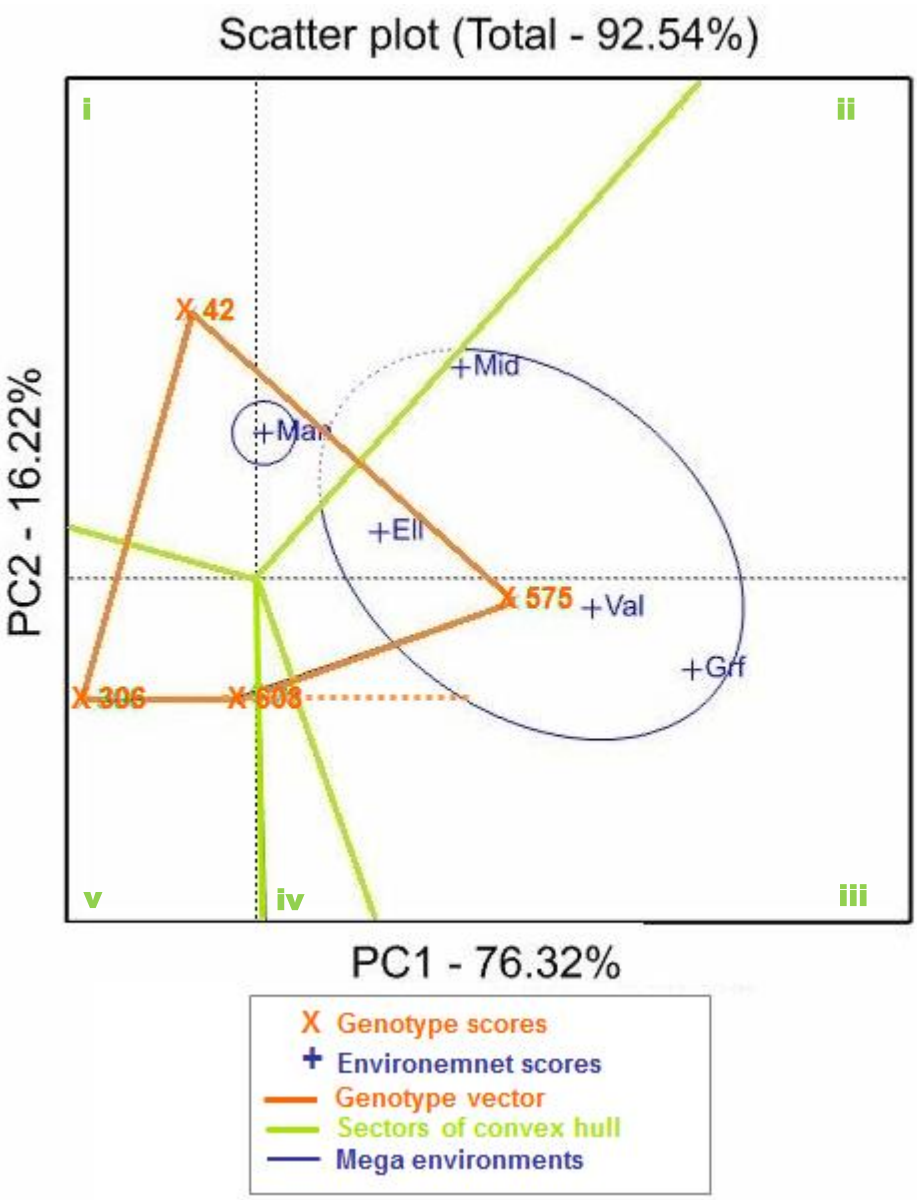

Figure 6.12 Convex hull (which-won-where) view of the GGE biplot showing the perpendicular that bisects the vertices of the convex hull (polygon) intersecting the invisible extension (broken line) of the line connecting two genotypes

No environments were associated with sector iv containing no genotypes or sector v containing 306 and 608 indicating that these rootstock genotypes were not the best in any of the environments (citrus scion types), in effect they were the poorest performing rootstock genotypes for all the environments (citrus scion types).

Since a mega environment is defined as a group of localities that consistently share the best set of genotypes, citrus scion types Mid, Ell Val and Grf formed one of the mega environments and Man formed the other. For mega environment identification, crossover interaction such as in Figure 6.11a should be repeatable over years. Figure 6.11a pertains to the combined data over five years. However, repeatability was confirmed by comparing the mega-environment comparison plots of each of the individual years (data not shown).

For convex hulls, the genotypes located on the vertex of the polygon were either the best or the poorest performers in one or more environments. Rootstock 575 was specifically adapted to the first group while the best rootstock for mandarin was rootstock 42. This view of the data is based on the inner product property of the biplot and is not influenced by different SVD methods. However, according to Yan and Tinker (2006) the environment-focused partitioning (SVP=2) is more appropriate as it shows the relationships among environments.

Figure 6.11a indicates that rootstocks could be grafted to individuals of any of the citrus scion types except mandarins when evaluating rootstocks for yield potential. This was tested by the polygon view for different citrus scion types over five years (Figure 6.13). As expected, rootstock 575 was identified as the winning rootstock in the Valencia, grapefruit and Ellendale citrus scion types and rootstock 42 was identified as the winning rootstock in most of the years for mandarins.

However, the midseason group did not react as expected and instead of 575 being the best performing rootstock for this group, 42 was identified in most of the years. However, it was observed that the ellipse that encapsulates the environments in sector ii to form a mega environment overlaps with sector i and Mid being almost on the sector line dividing sector i and ii of the convex hull explains this discrepancy.

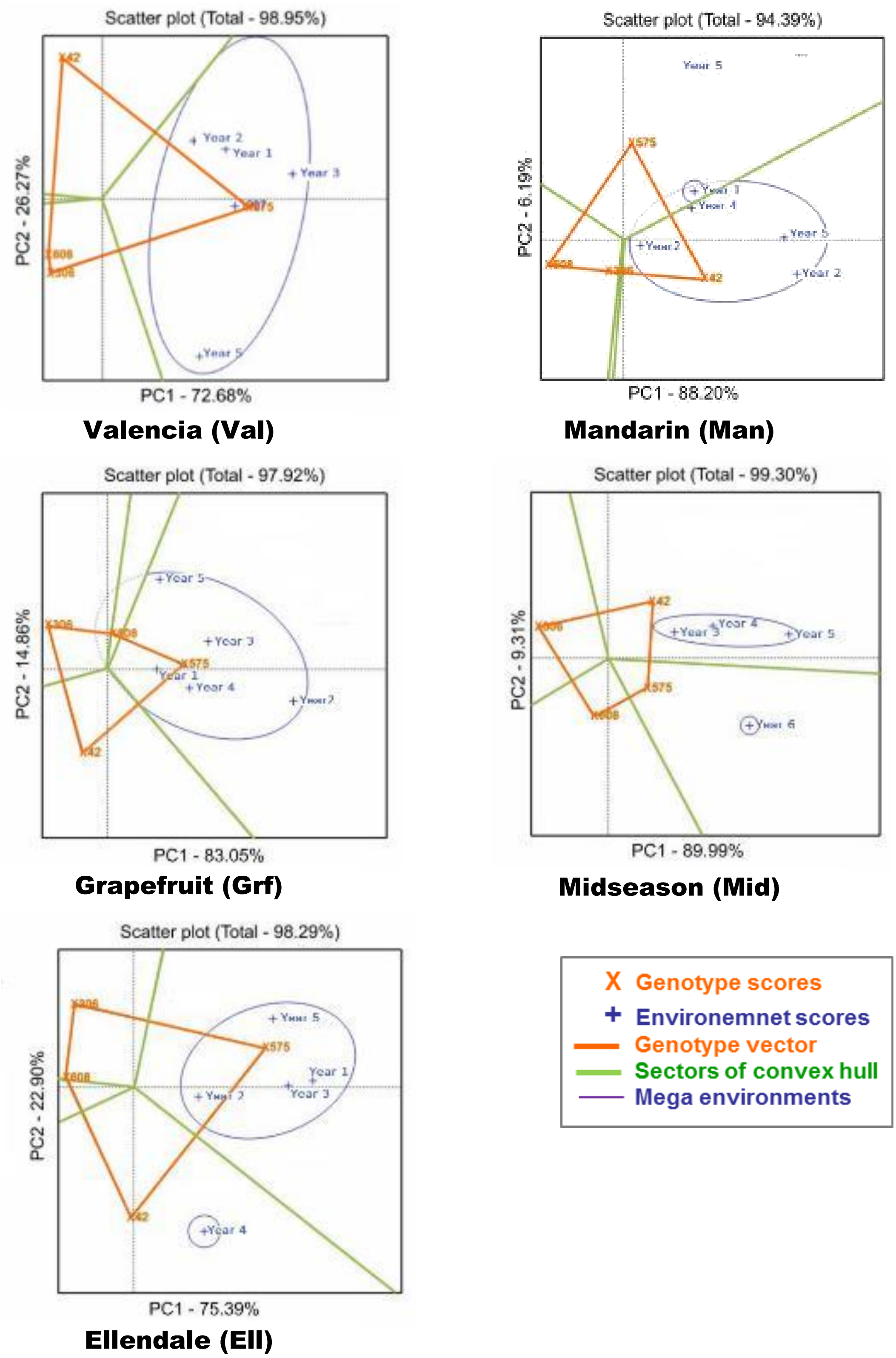

Figure 6.13 **Polygon (which-won-where) view of the GGE biplot to show which rootstock genotypes performed best within five different citrus scion types over five years**

6.3.2.3 Environment-vector view of GGE biplots for rootstock evaluation

Figure 6.14 is an environment comparison view, generated by Genstat 15 with regard to yield of four rootstock genotypes (G) evaluated with five different citrus scion genotype citrus scion types (E) based on an environment centred GEI table. This biplot explained 92.54% of the total variation of the environment centred GEI table and the results are as follows:

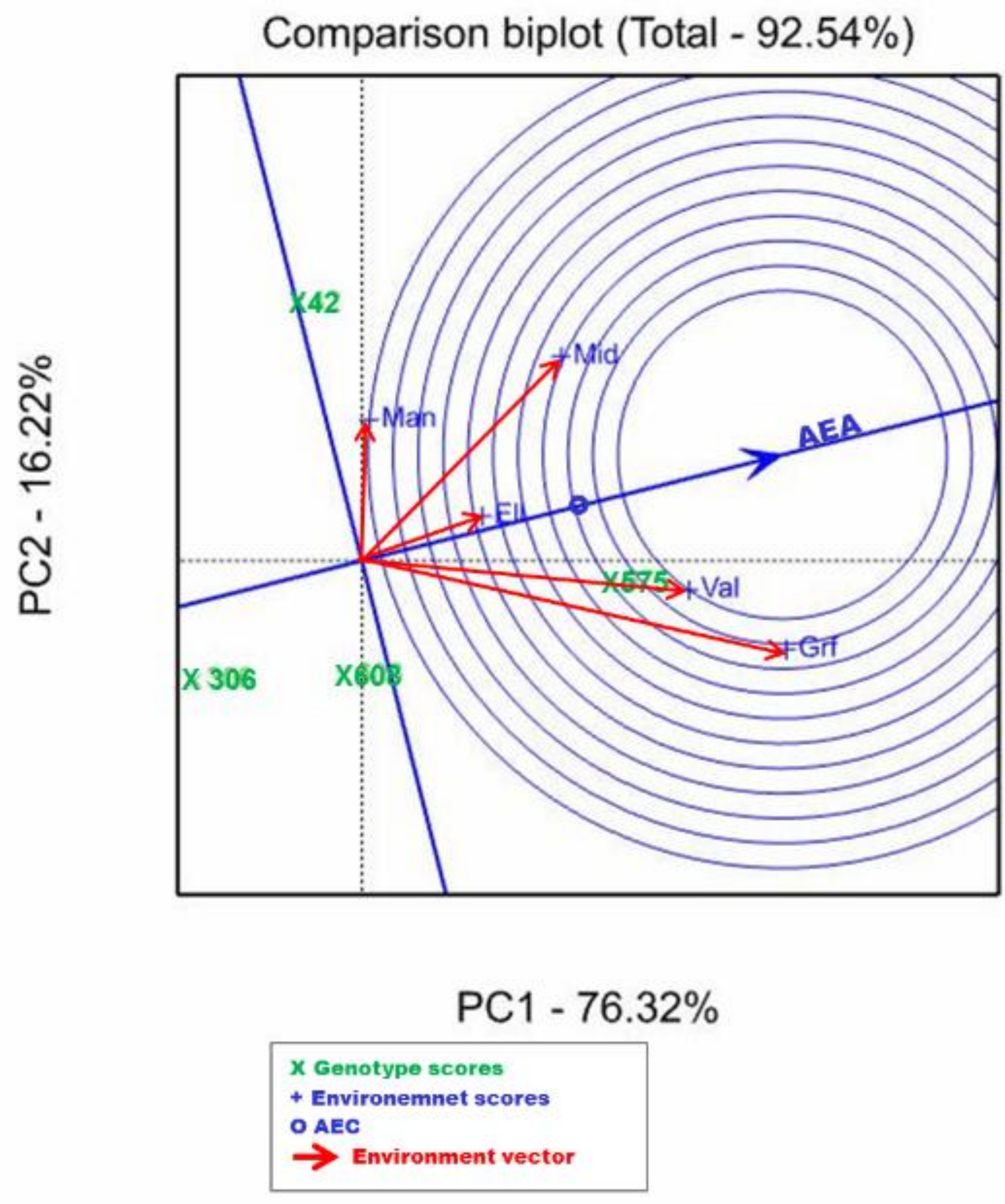

Figure 6.14 The environment-vector view of the GGE biplot of four rootstock genotypes (G) evaluated with five different citrus scion genotype citrus scion types (E)

Relation amongst test environments

i. According to Yan and Tinker (2006) the cosine angle between the vectors of test environments approximates the correlation between them. Val and Grf are thus positively correlated.

ii. The cosine angle between Man and Val and Man and Grf are slightly larger than 90°. These wide obtuse angles imply that Man is negatively correlated with Val and Grf indicating a compelling crossover GEI.

iii. The distance between two environments measures their dissimilarity in discriminating power with regard to the genotypes. Val, Grf and Mid thus formed a group based on their vector lengths, while Man and Ell formed another group.

Discriminating ability of test environments

The concentric circles on the biplot help to visualise the length of the environment vectors, which is proportional to the standard deviation within the respective environments (Yan and Tinker, 2006) and is a measure of the discriminating ability of the environments. Grf was thus the most discriminating while Ell and Man were the least discriminating environments.

Representativeness of test environments

A test environment that has a smaller angle with the AEA is more representative. Ell is thus the most representative of other test environments and Man the least representative.

6.3.2.4 Genotype genotype-vector view of the GGE biplot

Figure 6.15 is a genotype comparison view, generated by Genstat 15 and is based on a genotype centred GEI table of the same data as in section 6.4.1. This biplot is similar to the biplot in Figure 6.14 except that it is genotype-metric preserving (SVP=1) and is therefore appropriate for comparing genotypes. In this biplot the origin represents a "virtual" genotype that assumes the average value in each of the environments, thus having no inputs to G or GEI.

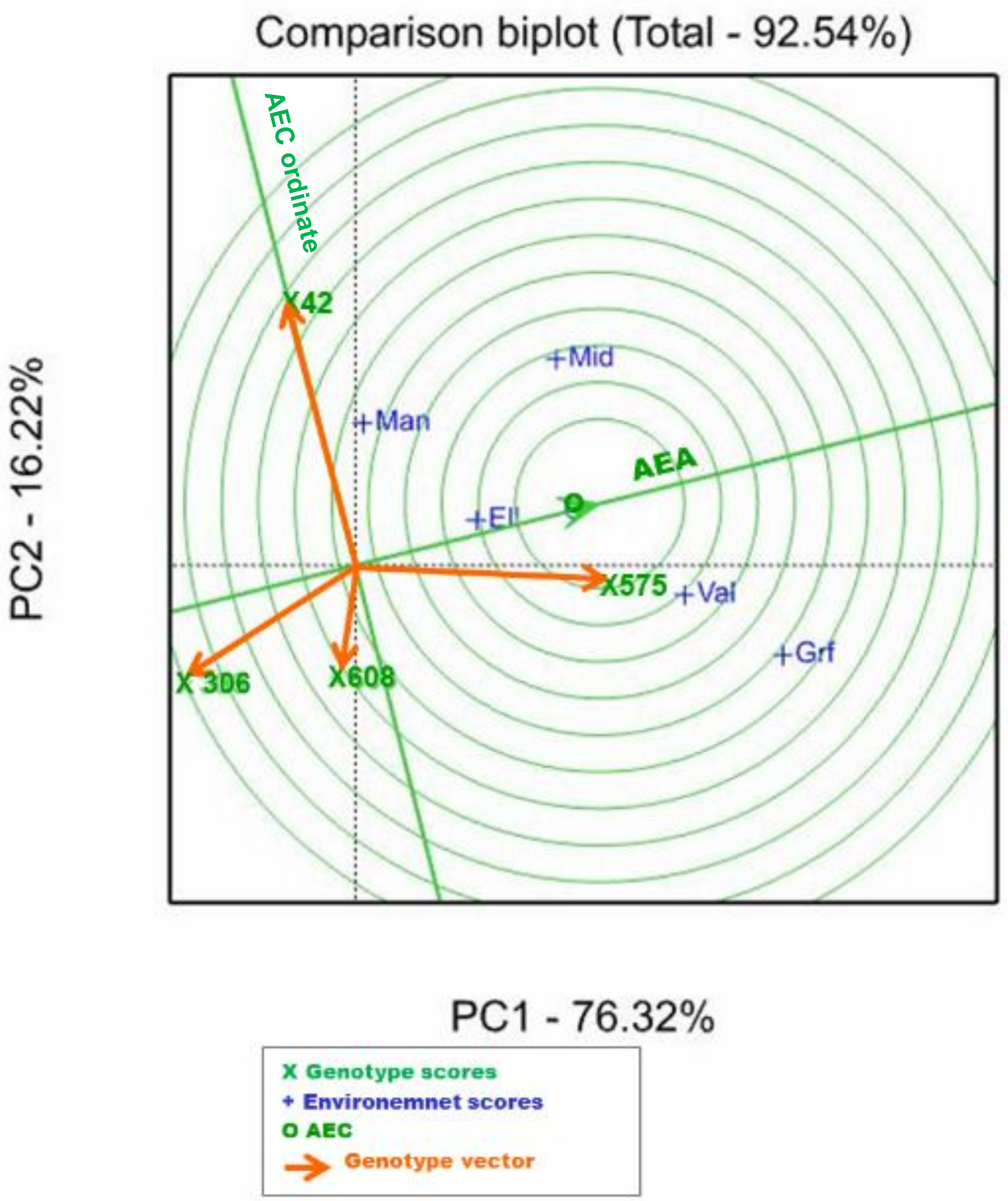

Figure 6.15 The genotype-vector view of the GGE biplot based on genotype focussed scaling for comparison of genotypes with the ideal genotype of four rootstocks genotypes (G) evaluated with five different citrus scion types (E)

An ideal genotype should have both high mean performance and stability across environments. In Figure 6.15 the ideal genotype is in the centre of the concentric circles. This biplot explained 92.54% of the total variation of the genotype centred GEI table and the results are as follows:

i. The angle between two genotypes indicates their similarity or dissimilarity in response to the environment. Dissimilarity could be attributed to either differences in mean yield (g) and/or interaction with the environment (GEI). An acute angle between rootstock genotype vectors (306 and 608) indicates that the two genotypes responded similarly and that the differences between them were proportional in all environments. An obtuse angle (42 with 575, 306 and 608) means that the two genotypes (42 and 575 or 42 with 306 or 42 with 608) responded in opposite directions, thus wherever the one performed well the other performed poorly. With regard to acute and obtuse angles, the differences contributed mostly to G. A right angle (575 with 608) indicates that the two genotypes responded to the environment independently and the difference in this case contributed mostly to GEI.

ii. The origin of the biplot represents a virtual genotype with an average value and the genotype vector measures the distance between the genotype and the origin. Therefore, the genotype vector represents the deviation of the genotype from the "average" genotype and thus gives an indication of its contribution to G or GEI. Longer vectors thus imply a larger G and GEI contribution and vectors in the direction of the AEA are either the best (575) or the poorest (306) with longer vectors in the direction of the AEC ordinate being more unstable (42) and genotypes in very close proximity of the origin have very little or no GEI.

iii. The angle between the genotype vector and the AEA partitions the genotype vector length into components of G and GE. A right angle with the AEA indicates that the contribution is mainly due to GEI (42) while an obtuse angle indicates that the contribution is mainly due to G (608) which leads to a below average mean performance and an acute angle means the contribution is mainly G which leads to an above average mean performance (575).

6.3.2.5 Mega environment analysis for scion evaluation

Based on the limited number of environments (four rootstocks) used in this study, four sectors and one mega-environment was formed (Figure 6.11b). Midseason, Valencia, mandarin and grapefruit were the four winning genotype groups in each of these sectors. Ellendale and mandarin were grouped together in a single sector. In this case, the rootstocks formed one mega-environment with grapefruit as the best performer at the vertex of the polygon. Figure 6.11(b) indicated that the discriminating ability of a best performing genotype could be evaluated grafted on any of the rootstocks. This is proven in the AMMI analysis (data not shown) where grapefruit, Valencia and Ellendale were consistently ranked one, two and three in association with each environment (rootstock).

As in AMMI analysis, mega-environment determination is followed by genotype evaluation and test-environment evaluation within a mega environment (Yan *et al*., 2007). With regard to scion evaluation, this will pertain to the genotypes within a group such as for e.g. the Valencia group.

Figure 6.16 represents the genotype-vector view of the GGE biplot based on genotype focussed scaling for comparison of yield of eight Valencia selections (G) on four rootstocks (E) at one locality. Figure 6.16 explained 89.82% of the total variation of the genotype centred GEI table and the results are as follows:

i. An acute angle between genotype vectors 1060, 1043a, 1056 and 1063 indicated that the two genotypes responded similarly and that the differences between them were proportional in all environments. The obtuse angle that Valencia selection 1053 formed with all of the other genotypes indicated that it responded inversely thus, wherever it performed well the other performed poorly and *vice versa*. Valencia genotypes 1043b and 1044 responded similarly but both responded inversely to the rest of the genotypes
ii. With regard to acute and obtuse angles, the differences were mostly due to G. The difference between Valencia genotypes 1063 and 1043b is thus genetic while the difference of yield between Valencia genotypes 1063 and 1052 is mostly due to GEI as the near right angle between the two genotypes indicates that the two genotypes responded to the environment independently and the difference in this case contributed most to GEI.
iii. Longer vectors imply a larger G and GEI contribution and vectors in the direction of the AEA are either the best (1063 and 1056) or the poorest (1043b and 1044). Longer vectors in the direction of the AEC ordinate are more unstable (1052) and genotypes in very close proximity of the origin (1055) have very little or no contribution to GEI.

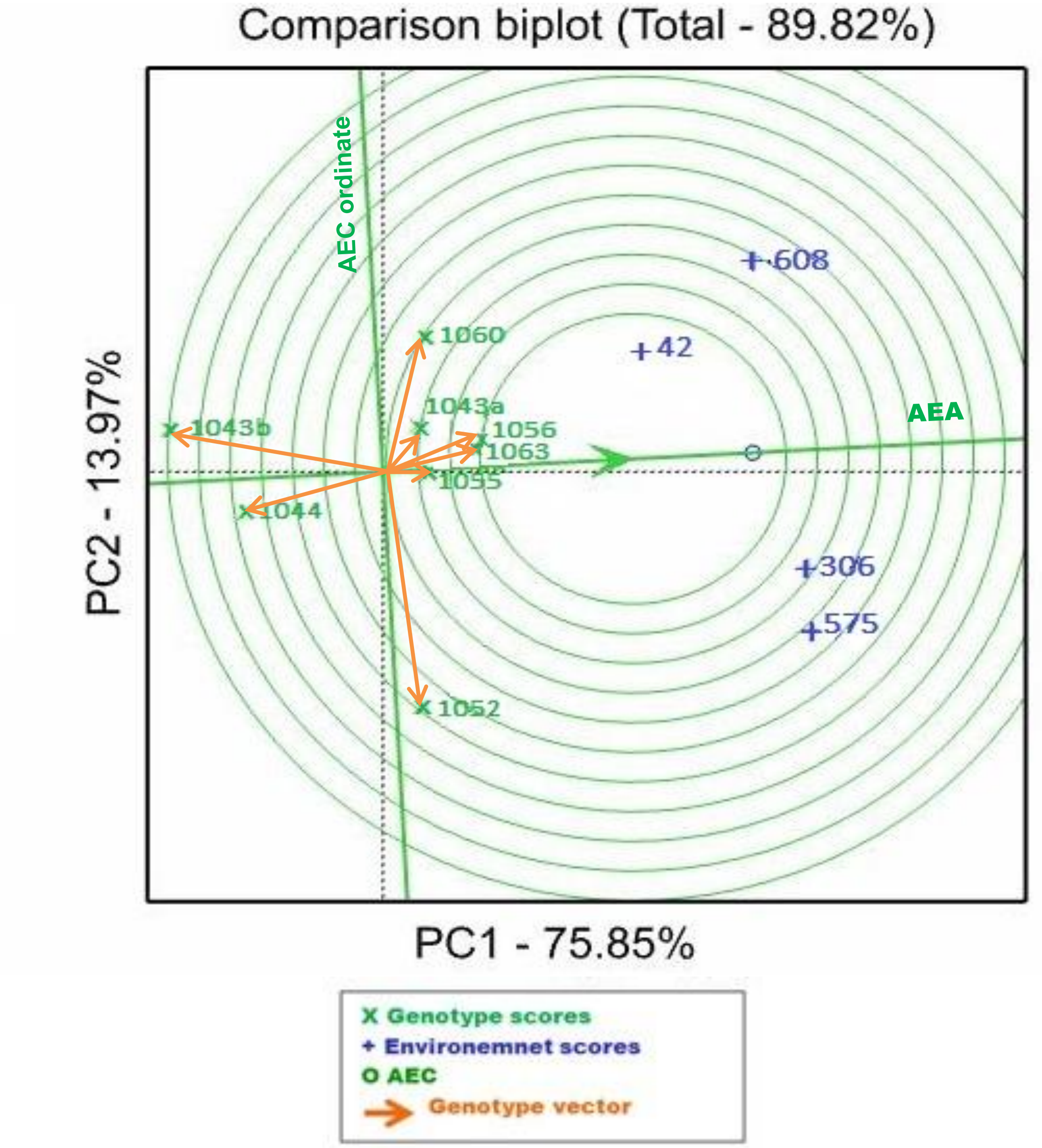

Figure 6.16 The genotype-vector view of the GGE biplot based on genotype focussed scaling for comparing yield potential of eight Valencia selections (G) on four rootstocks (E) at one locality

Figure 6.17 represents the same data than Figure 6.16 but with years as environments. Figure 6.17 explained 89.82% of the total variation of the genotype centred GEI with genotypes 1052 and 1063 having the same angle and genotypes 1055 and 1060 having nearly the same angle. This indicates that the two genotypes responded similarly and that the differences between them were proportional in all years. However, 1063 and 1052 were very close to the AEA and therefore stable whereas 1055 and 1060 were more prone to GEI. Obtuse angles of genotypes 1043b, 1044 and 1043a with regard to the other genotypes indicated that these three genotypes performed poorer than average and responded inversely to the rest of the genotypes.

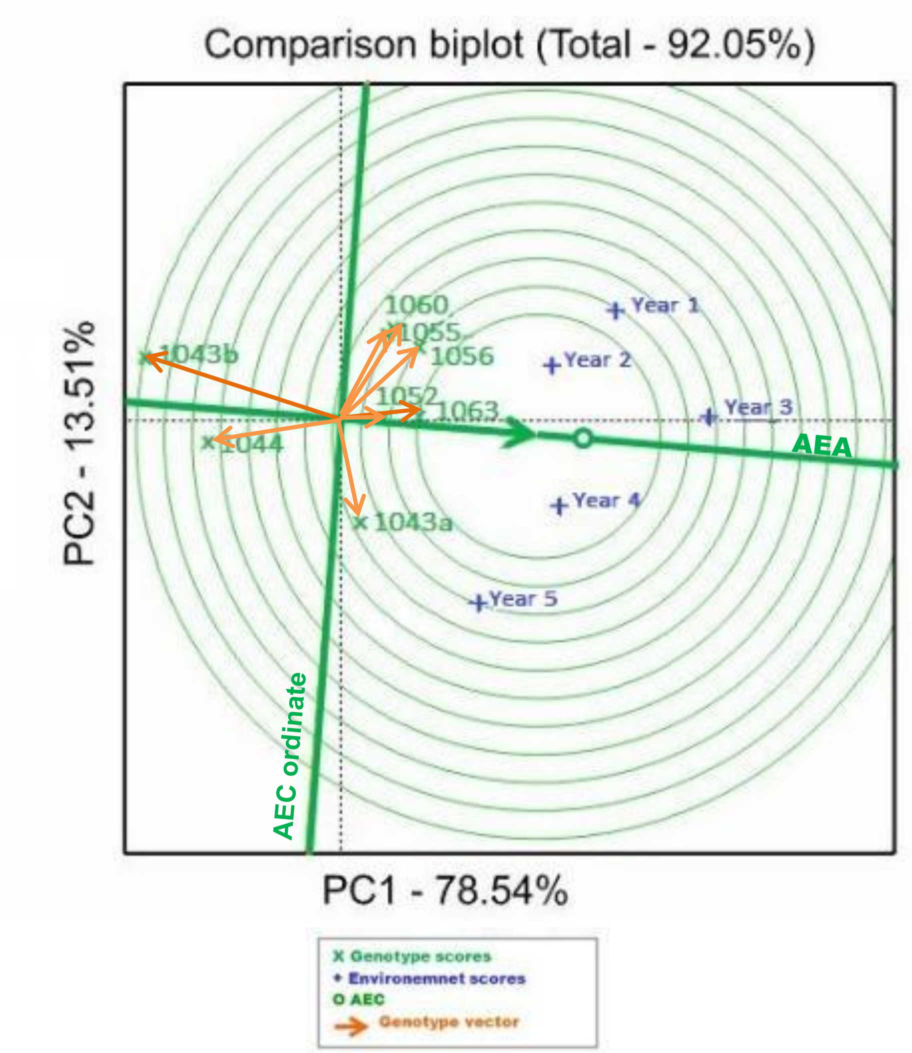

Figure 6.17 GGE biplot of variance for yield of eight Valencia selections (G) evaluated for five consecutive years (E) on four rootstocks at one locality

Where Figure 6.17 illustrated the scion by year effects, Figure 6.18 illustrates the rootstock by year effects. Longer vectors imply a larger G and GEI contribution and vectors in the direction of the AEA are either the best (575) or the poorest. Longer vectors in the direction of the AEC ordinate are more unstable (42) and genotypes in very close proximity of the origin (575) make very little or no contribution to GEI. Figure 6.18 thus shows rootstock 575 to have the best as well as stable mean performance over years.

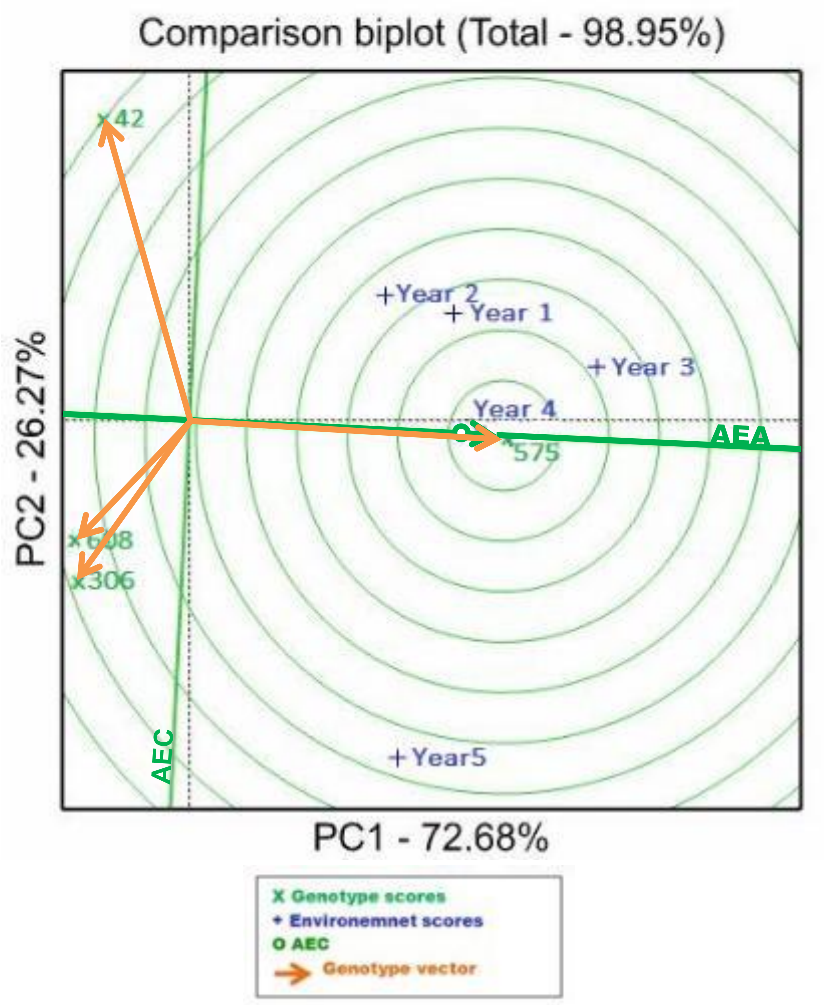

Figure 6.18 GGE biplot of variance for yield of rootstock (G) genotypes evaluated for five consecutive years (E) at one locality with eight selections (E) within the Valencia group

6.3.3 The mean vs. stability coordination view of the GGE biplot

Figure 6.19 represents the mean vs stability view of the GGE biplot and can be used to facilitate genotype comparisons based on mean performance and stability across environments within a mega-environment.

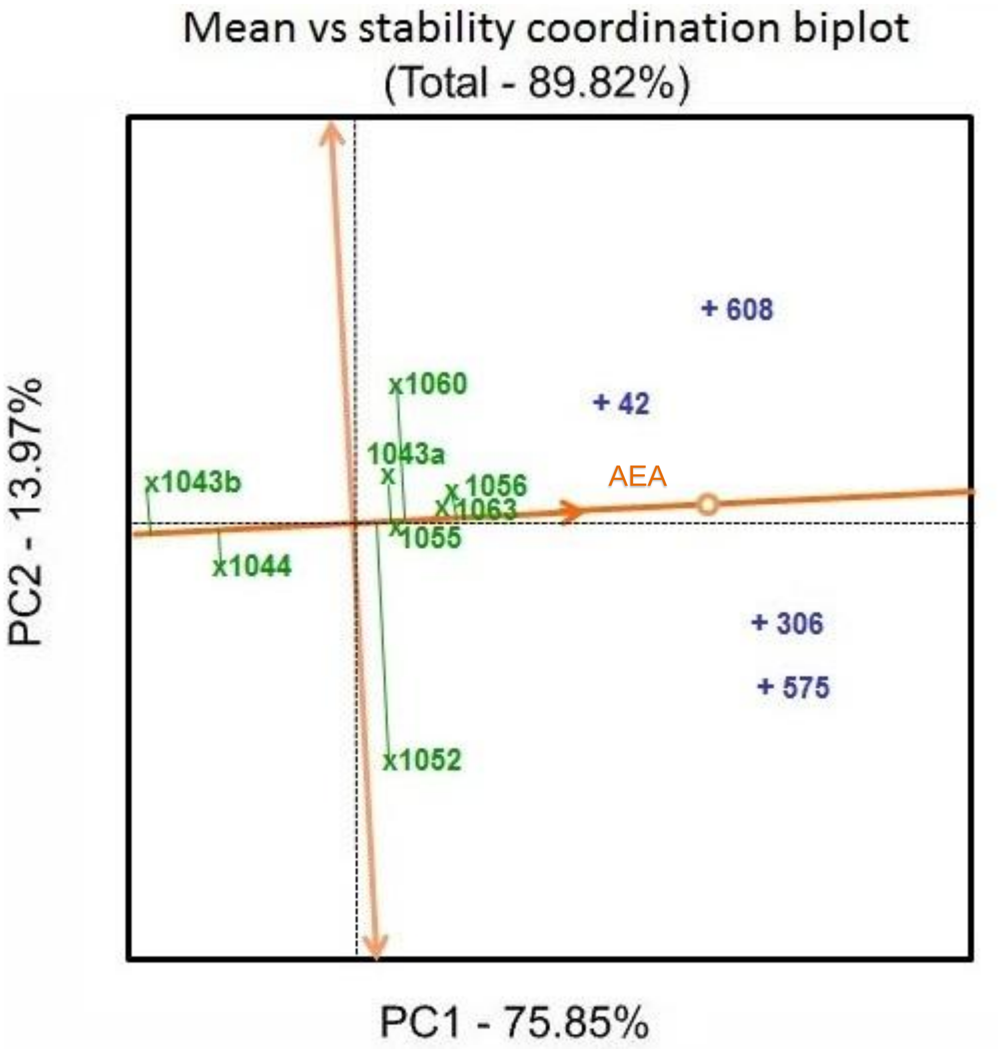

Figure 6.19 Mean vs stability coordination view of the GGE biplot based on genotype focussed scaling for comparison of eight Valencia selections (G) on four rootstocks (E) at one locality within the Valencia group

The mean vs stability view projections of the genotype markers on the AEA represent the main effects of the Valencia genotypes (G) with the arrow of the AEA pointing in the direction of higher mean performance thus ranking Valencia selection 1056 the highest and 1043b the lowest. An increased vertical distance from the AEA parallel to the AEC ordinate, thus the length of the projection line indicates a greater tendency for GE interactions of the genotype and therefore less environments deeming Valencia selection 1052 the most unstable. Likewise, genotypes that cluster together on the AEA with a short projection on the AEA is highly stable and would thus perform consistently across environments such as Valencia selections 1063, 1055 and 1056.

6.4 CONCLUSION

This investigation proved that by using GGE biplots and the biplot function of the AMMI model a more complete and visual evaluation of all aspects of the data was possible. Fast and accurate data interpretation was possible using biplots that identify mega-environments and that can simultaneously represent both mean performance and stability within a mega environment.

Information gained from GGE biplots mostly concurred with that from the AMMI biplot as both models apply the same mathematical principals. However, where slight deviations were found it could be attributed to the GGE method applying SVD to the data after the environment means have been removed, while AMMI applies SVD after both the genotype and environment means have been removed. AMMI is thus able to clearly distinguish between the main and interaction effects whereas separation of G from GE is a mathematical impossibility for GGE analysis. This was a very important feature in the separation of rootstock effect from that of the stion in Chapters 4 and 5.

Visual analysis with the AMMI biplot was relatively simple and was found to be beneficial in identifying mega-environments, estimating the magnitude and significance of GEI and to determine general and specific adaptability of genotypes in relation to the test environments. GGE was good at defining mega environments as well as the relation amongst test environment and could determine the discriminating ability as well as representativeness of test environments which was not possible in the AMMI.

It was also found that the AMMI model could determine the ability of genotypes to have the same relative performance in different environments through the AMMI stability value which is calculated directly from the genotype's contribution to the interaction sum of squares.

It was found that neither the AMMI nor the GGE biplots could visualise the relative performance of genotypes in an environment or group of environments but do not provide any measure of the actual yields or other traits. However, the AMMI model does provide statistics as can be seen in Tables 6.1 to 6.6 and the two models therefore complement each other and should be used simultaneously for interpretation of GEI.

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Chapter 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 CONCLUSIONS

The success of a commercial plant-breeding programme depends on its ability to provide farmers with genotypes having guaranteed superior performance in terms of yield and/or quality. Farmers are primarily concerned about the long-term performance of new genotypes in their production environments. A breeder, on the other hand, wants to select for consistently high performance over all environments. In order to improve genotype performance, researchers other than breeders such as horticulturists and soil scientists are concerned with improving or manipulating the environment in which the genotype functions. In the equation P = G + E + GEI the breeder is thus more concerned with the G and GEI while the other scientists are more concerned with E and GEI.

According to Jacobsen *et al*. (2013) the best strategy to keep pace with global population growth and increasing food demand is constantly under debate among researchers with one strategy favouring the use of genetically modified (GM) crops, while another strategy focuses on agricultural biodiversity. However, it is irrelevant who is right or wrong and whether selections are the product of genetic engineering or conventional breeding, field trials are and will be the main source of information about the behaviour of new selections and cultivars.

Another important factor in a successful commercial breeding programme is the financial input needed to develop new selections to farm ready cultivars. It does not matter anymore whether it is a private or public venture as both in one way or another calculates the return on investment that a new cultivar will present first for the breeder and secondly for the industry. Due to the GEI in the above-mentioned equation, field trials are conducted under different environmental conditions, i.e. varied crop management, and soil and climate conditions to become multi-environment trials with the additional impact of cost to the breeding programme. The financial impact of this kind of trials is more pronounced in fruit tree breeding than in crop breeding. It is, therefore, vital that the statistical methods used to design and analyse data from crop cultivar breeding and evaluation programmes are as accurate, efficient, and informative as possible.

The most recent factor that will definitely affect the success of a commercial plant-breeding programme is the impact of global warming and climate change. Future fruit breeding programmes should therefore focus on cultivar resilience rather than the breeding of merely high performing cultivars.

Given the prerequisites for the success of newly bred cultivars, this study was initiated to conduct a systematic analysis of the GEI through application of novel approaches that may add value to future citrus METs. The classical approach to analysis of field trials subjects data to an ANOVA followed by one of the t-comparison tests, such as Student t-test, Tukey's or Duncan's Multiple range test, to separate the means. The ANOVA techniques even partition total variation into sources due to selections, environments (locality/year combinations), GEI and within-trial error variation but is unable to explain GEI (Odewale *et al*., 2013) as was illustrated in chapter 3.

From a breeder's point of view, merely obtaining an estimate of overall (average) selection/cultivar performance across environments, may impede the selection process during breeding and/or selection/cultivar recommendation following a field trial. With the need for future fruit breeding programmes to focus on cultivar resilience, a measure of varietal stability to environmental change may be required. This can either be for the identification of selections/cultivars that are both high yielding and stable (so are suitable for broad use) or varieties that perform exceptionally well under certain conditions (so may be suitable for use in specific environments).

In a composite grafted plant, rootstocks control many aspects of scion growth and physiology including yield and quality attributes as well as biotic and abiotic stress tolerance. Thus in tree crops that are dependent on grafting, phenotypic response is specific in relation to stionic combinations used, and new hybrids must be evaluated for these reactions. A grafted or budded plant can produce growth patterns and reactions which may be different from what would have transpired if each part of the stion were grown separately or when the scion was grafted or budded in other types of rootstocks. Thus, even when a scion evaluation trial is conducted on a single rootstock, recommendation of the best scion selection for future cultivar status or adaptability to a specific locality is always conditional. Due to the interaction between rootstocks and scions, even horticultural trials such as for pruning, irrigation or fertiliser can pose a problem if the G, E and GEI of the scion and rootstock cannot be separated.

In citrus breeding, determining the breeding value of available parents and the heritability of specific characters can be a great aid to the breeder in predicting which parent combinations will produce superior progeny (Ray, 2002). Determining heritability is already cumbersome in breeding of tree crops due to the heterogeneous nature of the crop and the lack of breeding progenies due to the costs involved. According to Yan and Kang (2003) GEI, as components of total phenotypic variance, affect heritability (proportion of total phenotypic variance that is due to genetic variance). The larger the GEI component is, the smaller the heritability estimate thus, progress from selection would be reduced as well. According to Gauch (2006) GEI decreases heritability given a single target region, but can largely be mitigated by appropriate mega-environments. To further compound the problem, the phenotype pertains to a single (but composite) plant where the G, E and GEI pertains to two genotypes within this plant.

The applicability of AMMI analysis was illustrated in Chapters 4, 5 and 6 to:

i. partition the phenotype (P) of the stion into its G, E and GEI components,
ii. further partition the phenotype into a scion G, E and GEI as well as a G, E and GEI for the same phenotype as that of the stion,
iii. determine test environments. It was also determined that test environments for breeding purposes should be highly discriminating and for cultivar evaluation an "ideal" test environment should be both discriminating of the genotypes and representative of the mega-environment,
iv. based on stability, effectively rank genotypes that did not differ significantly from each other in t-tests,
v. AMMI produces biplots with a single set of interpretive principles rather than numerous different cases and AMMI is uniquely the statistical method among SVD-based analyses that treats G, E, and GEI separately
vi. understanding interactions and implementing mega-environments can be strategic, accessing several times as much genetic variability for yield and other important traits and
vii. because the challenges and opportunities presented to breeders by G differs from that GE, it is desirable for statistical analysis to address both, but separately. Likewise, for horticulturists, it is desirable that statistical analysis captures but also distinguishes E and GE. Consequently, to serve the needs of all agricultural

researchers, the best statistical analyses must distinguish G, E, and GE and this is possible with AMMI analysis.

The applicability of GGE biplots was illustrated in Chapter 6 with regard to mega environment delineation, genotype evaluation within a mega environment and relation amongst test environments as well as the discriminative and representativeness of the various environments. It was found that generating GGE biplots was relatively easy, quick through the application of GENSTAT software. The visual attributes of GGE biplots are clear, appealing and easy to interpret by applying a few standard interpretation rules.

7.2 RECOMMENDATIONS

- The inability to evaluate statistical significance of genotype and environment co-ordinates on the biplot is one of the limitations of GGE biplots (Yang *et al*., 2009). Therefore, despite visual attributes of GGE biplots being more favourable than that of AMMI, the preferred method of interpreting interactions in this study was AMMI analysis due to the statistics generated such as the ANOVA table and the ASV.
- Although Gauch (2006) stated that AMMI analysis was superior to GGE biplots and that there is no call for a mix-and-match strategy using both methods, its use in other citrus G by E studies is still recommended as long as it is verified by an AMMI analysis. GGE biplot analysis allowed for quick, clear and appealing visual illustrations and examinations with regard to mega environments delineation, ranking of genotypes and identification of test environments in this study which is conducive to publications.
- It is also recommended that already analysed and even published data from trials not necessarily planned as METS be scrutinised to see whether the layouts of the trials conform to a randomised block design and whether the data is suitable for an AMMI analysis. Re-analysis of old citrus data could affect the planning of future trials, selection of breeding parents as well as supply information on target regions and possible mega-environments.
- Investigations expanding on this study include the following
    - ❖ **The consequence of tree age at harvest**: Trials are sometimes planted with three to four months and even six months lapsing in between causing the tree age to differ. Data are being explored to investigate the accuracy of comparing these trials with each other.

- **Indirect selection** reduces the amount of measurements/observations having a positive resource impact on the breeding programme. According to Yan and Tinker (2006) the basic structure of MET data is actually a genotype-environment-trait three way table which can be organised into various two-way tables, which can then be studied in biplots to graphically address various questions. Genotype by trait biplots can help identify traits that are correlated (negative or positive) and that can be identified through indirect selection.
- **Probing the impact of climate changes in an environment:** Insight in the trait by environment interactions and environmental correlation amongst traits can be derived from environment by trait tables that are generated from genotype-environment-traits three way tables. According to Yan and Tinker (2006) this type of analysis might be of more importance to horticulturists but in the face of climate change this can become a tool of determining the actual impact of environmental change on a trait and even predicting genotype-environment reactions.
- **Marker assisted selection and QTL identification**: According to Yan and Tinker (2005) the G and GE of a target trait such as yield can be interpreted in terms of covariate or explanatory traits. When the genetic covariates are markers the QQE or the genetic marker by environment plot can be used to identify genetic regions that are associated with traits in one or more environments (QTL identification) and assist in marker-assisted selection specific to different mega environments.
- **Detailed physiological and crop modelling research:** Ramburan (2012) have proved that the pattern analysis and grouping strategies made possible by AMMI and GGE analysis have potential beyond conventional G by E research, as it can assist in selecting representative genotypes (and environments) for detailed physiological and crop modelling research.
- **Appropriateness of the HA-GGE biplot for visual evaluation of the test environments:** Yan and Holland (2010) introduced a heritability-adjusted (HA) GGE biplot that graphically ranks test environments, compared to a collective target environment, based on their $r\sqrt{H}$ values.

The main consequence of applying multivariate models is the simplified and thus cost effective trial layout that it facilitates. There is no need any more for separate rootstock and scion trials. These trials can now be combined, incorporating selections from both the scions and rootstock programmes, saving orchard space and time but generating more information. The envisaged outcome of the study is a statistical method that in future trials would enable breeders to recommend the best rootstock-scion combination from time and cost effective evaluation of promising selections from the South African citrus breeding programme.

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SUMMARY

Cultivars with high and stable genetic potential for production and quality is the main goal of a fruit-breeding programme and are assessed in multi-environment trials due to the influence of rootstocks as well as climate on yield and quality in citrus. In a crop where grafting is essential, the manifestation of the scion's genotype is dependent on the rootstock on which it is grafted as well as the environment in which the scion-rootstock combination (stion) is grown. The problem with grafted trees is that part of what is measured in these trials is the rootstock's reaction to the environment as well as the rootstock's interaction with the scion and vice versa, which constitutes complex genotype x environment interactions (GEI). The aim of this study was to successfully separate the Genotype (G) and GEI of the stion into a scion and a rootstock G and GEI.

Data used in this investigation emanated from Phase II trials within the South African citrus breeding programme and comprised of five citrus scion types namely grapefruit (*Citrus paradise)*, midseason oranges *(C. sinensis*), Valencia oranges (*C. sinensis)* early mandarins (*C. reticulate*) and late mandarins (*Citrus paradise*). Rootstock selections that were included were: Van Stadens Rough lemon (*C. jambhiri*, Tenaka), Volckamer lemon (*C. volkameriana* V. Ten. & Pasq.), Empress Rosehaugh (*C. reticulate* Swingle) and Carrizo citrange (*Poncirus trifoliate* x *C. sinensis*). Three localities were included namely Messina, Malalane and Friedenheim for the univariate study. Data from Malalane over five years was used to test two multivariate models namely AMMI and GGE.

The multivariate analysis confirmed citrus scion types as mega environments in relation to rootstocks. No single mega environment for rootstock selection, that was both discriminative and stable with regard to all the traits in this trial, could be found. Mega environments were trait specific with, for instance grapefruit, representing the ideal test environment for peel thickness and Valencia the most stable environment with regard to TSS:TA ratio. It was also found that the AMMI model was able to separate and quantify the contribution of the scion and rootstock to the stion in a single physical environment (i.e. same climate, same soil, same production practices). As was expected, the scion contribution was found to be more prominent than the rootstock contribution for most of the traits. There were exceptions such as in grapefruit, where the rootstocks were responsible for 53.21% of the yield effect as opposed to the 27.50% contribution of the

scion and 54.96% with regard to peel thickness opposed to the 38.23% of the scion. GEI regarding scion (G) x rootstock (E) was significant but not for rootstock (G) x scion (E). This implies that the rootstocks in some or another way influenced the scions whereas the scions had no significant influence on the rootstocks.

With a good insight into the biplot theory, the interpretation of the visual aspects of both the AMMI and GGE were found to be easy and beneficial. A dataset can generate a multitude of graphs which can render information at a quick glance but still with scientific context.

The main consequence of applying multivariate models is the simplified and thus cost effective trial layout that it facilitates. There is no need any more for separate rootstock and scion trials. These trials can now be combined, incorporating selections from both the scions and rootstock programmes, saving orchard space and time but generating more information. The envisaged outcome of the study is a statistical method that in future trials would enable breeders to recommend the best rootstock-scion combination from time and cost effective evaluation of promising selections from the South African citrus breeding programme.

OPSOMMING

Sitrus cultivars met 'n hoë en stabiele genetiese potensiaal vir produksie en kwaliteit is die hoof doelwit van 'n vrugte teelprogram en moet op verskillende onderstamme asook verskillende klimaatsomgewing geëvalueer word en word multi-omgewings (MO) proewe genoem.

In 'n gewas waar klonale voortplanting nodig is vir tipe-egtheid en siekte beskerming en voortplanting dus afhanklik is van 'n onderstam, moet die interaksie van die bostam met die onderstam in ag geneem word. Die probleem met geënte bome in MO proewe, is dat 'n deel van wat gemeet word, die onderstam se reaksie op die omgewing sowel as die onderstam se interaksie met die bostam - en omgekeerd met ander woorde 'n komplekse genotipe x omgewing interaksies (GEI) . Die doel van hierdie studie was om die genotipe (G) en GEI van die geënte plant ("stion") suksesvol te skei in 'n bo-en 'n onderstam G en GEI .

Data vir hierdie ondersoek is verkry uit Fase II proewe in die Suid- Afrikaanse sitrus teelprogram en bestaan uit vyf bostam groepe naamlik pomelo, midseisoen en Valencia lemoene asook vroeë en laat mandaryne. Onderstamme het ingesluit Van Stadens growweskil suurlemoen (*C. jambhiri*, Tenaka), Volckamer suurlemoen (*C. volkameriana* V. Ten. & Pasq.), Empress Rosehaugh (*C. reticulate* Swingle) en Carrizo citrange (*Poncirus trifoliate* x *C. sinensis*). Drie lokaliteite naamlik Messina, Malalane en Friedenheim het deel uitgemaak van die studie. Data van Malalane oor vyf jaar is gebruik om twee meerveranderlik variansieanalise modelle naamlik AMMI en GGE te toets.

Die meerveranderlik variansieanalise bevestig sitrus groepe as mega-omgewings met betrekking tot onderstamme. 'n Enkel geskikte mega-omgewing vir onderstam seleksie, wat diskriminerend maar terselfdertyd stabiel was ten opsigte van al die eienskappe, kon nie gevind word nie. Mega-omgewings was spesifiek ten opsigte van eienskappe soos byvoorbeeld pomelo, wat as die ideale bostam groep geïdentifiseer is om skildikte te toets en Valencia as die mees stabiele omgewing met betrekking tot die verhouding van totale oplosbare stowwe tot titreerbare suurinhoud. Daar is ook bevind dat die AMMI model in staat was om in 'n enkel lokaliteit onderskeid te tref ten opsigte van die bo- en onderstam bydrae tot die fenotipe van die geënte plant. Soos verwag het die bostam 'n meer

prominente bydrae gelewer vir meeste van die eienskappe, as die onderstam. Daar was egter interessante uitsonderings soos byvoorbeeld vir pomelo, waar die onderstamme verantwoordelik was vir 53.21% van die produksie teenoor die 27.50% bostam en 54.96% met betrekking tot skildikte teenoor die 38.23% van die bostam. GEI was betekenisvol waar die onderstam as ’n omgewing vir die bostam beskou was maar was nie betekenisvol in die omgekeerde situasie nie. Dit beteken dat die onderstam in die geval die bostam beïnvloed het terwyl die bostam geen wesenlike invloed op die onderstam gehad het nie.

Met 'n goeie insig in die teorie van biplots, was die interpretasie van die visuele aspekte van beide die AMMI en GGE modelle maklik en voordelig gewees. ’n Enkele datastel het 'n magdom grafieke gegenereer. Elkeen van die grafieke kon met slegs ’n oogopslag inligting beskikbaar stel, sonder om die wetenskaplike konteks te verloor.

Die belangrikste gevolg van die toepassing van meerveranderlik variansieanalise modelle is die vereenvoudigde en dus koste-effektiewe proef uitleg wat dit fasiliteer. Afsonderlike bo- en onderproewe is nou onnodig. Hierdie proewe kan nou gekombineer word en instede van om onderstamseleksies net met ‘n enkele standaard bostam te ent, kan kombinasies van bo en onderstam seleksies saam met kontroles in een proef ingesluit word wat dus vinnig baie meer inligting beskikbaar stel. Die beoogde uitkoms van die studie is 'n statistiese metode wat in die toekoms sitrustelers in staat sal stel om op grond van tyd en koste-effektiewe proewe, aanbevelings rondom nuwe belowende seleksies uit die Suid-Afrikaanse sitrus bo- en onderstam teelprogram te maak. Die aanbevelings sal gebaseer wees op stabiliteit sowel as die beste bostam-onderstam kombinasies.