# DEVELOPING A BREEDING STRATEGY FOR BUTTERNUT SQUASH (*CUCURBITA MOSCHATA*) IN SOUTH AFRICA

By

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**Declaration**

"I, Jacobus Francois Swanepoel, declare that the thesis that I herewith submit for the Doctoral Degree Philosophiae Doctor in Plant Breeding at the University of the Free State, is my independent work, and that I have not previously submitted it for a qualification at another institution of higher education".

Jacobus Francois Swanepoel Date: 30 November 2021

## Dedication

This work is dedicated to my wife, Leanie

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## List of abbreviations

| | |
|---|---|
| °Brix | Sugar content of an aqueous solution |
| AEA | Average environment axis |
| AEC | Average environment coordinate |
| AFM | Average fruit mass |
| AMMI | Additive main effects and multiplicative interaction |
| ASV | AMMI stability value |
| ANOVA | Analysis of variance |
| CHL | Chlorophyll content |
| CV | Coefficient of variation |
| df | Degrees of freedom |
| DMC | Dry matter content |
| DMY | Dry matter yield |
| E or Env | Environment |
| ECV | Environmental coefficient of variation |
| $F_1$ | First filial generation |
| FAO | Food and Agriculture Organisation of the United Nations |
| FN | Fruit number |
| Fruit a* | Green-red colour contribution in the mesocarp |
| G | Genotype |
| GA | Genetic advance |
| GAM | Genetic advance as a percentage of the mean |
| GCA | General combining ability |
| GCV | Genotypic coefficient of variation |
| GGE | Genotype main effect plus genotype x environment interaction |
| G x E | Genotype x environment |
| HSB | Hue, saturation, brightness |
| $h^2_{bs}$ | Broad-sense heritability |
| $h^2_{ns}$ | Narrow-sense heritability |
| IBD | Internal breakdown of fruit mesocarp |
| IPCA | Interaction principle component axis |
| IPCA1 | Interaction principle component axis 1 |
| IPCA2 | Interaction principle component axis 2 |
| kg | Kilogram |
| *L*a*b** | Luminance and intensity of the colour space values |
| Leaf a* | Green-red colour contribution in leaves |

| | |
|---|---|
| Leaf b* | Yellow-blue colour contribution in leaves |
| LSD | Least significant difference |
| LW | Leaf width |
| mm | Millimetre |
| MPH | Mid-parent heterosis |
| MS | Mean square |
| $MS_e$ | Residual mean square |
| $MS_g$ | Genotypic mean square |
| PC | Principal component |
| PCA | Principal component analysis |
| PCV | Phenotypic coefficient of variation |
| PEN | Penetrometer reading |
| PL | Petiole length |
| PvsC | Parents *versus* crosses |
| $R^2$ | Coefficient of determination |
| $r_p$ | Phenotypic correlation coefficient |
| $r_g$ | Genotypic correlation coefficient |
| RASV | AMMI stability value ranking |
| RGB | Red, green and blue colour space values |
| RYSI | Yiled stability index ranking |
| SE | Standard error |
| SCA | Specific combining ability |
| Spp. | Species |
| TSS | Total soluble solids |
| Uniform | Uniformity |
| USA | United States of America |
| YSI | Yield stability index |
| $\sigma^2_A$ | Additive variance component |
| $\sigma_p$ | Phenotypic standard deviation |
| $\sigma^2_e$ | Environmental variance component |
| $\sigma^2_D$ | Dominance variance component |
| $\sigma^2_g$ | Genetic variance component |
| $\sigma^2_{gca}$ | General combining ability variance component |
| $\sigma^2_{gl}$ | Genotype x environment interaction variance component |
| $\sigma^2_p$ | Phenotypic variance component |
| $\sigma^2_{sca}$ | Specific combining ability variance component |

**Abstract**

Knowledge about inheritance mechanisms of economically important traits and the influence of environmental factors on their expression are crucial for the formulation of an appropriate breeding strategy in any crop. Currently limited information is available on this subject in butternut and even more so with regard to internal fruit quality characteristics. The aim of this study was to design an effective breeding approach to improve butternut internal fruit quality, without sacrificing yield. The aim was extended with the objectives to quantify phenotypic and genotypic variability in 42 genotypes for characteristics across environments and confirm stability in high-performing individuals, and to identify characteristics showing the greatest potential for improvement through estimating the genetic parameters and interrelations between these characteristics. From heterosis studies, it could be confirmed which characteristics should be improved through hybridisation. From the combined analysis of variance on 15 morpho-agronomic and internal fruit quality characteristics over three locations and two seasons, highly significant differences ($p \leq 0.001$) were observed between genotypes for all traits including leaf chlorophyll content (CHL), green-red (Leaf a*) and yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW), petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity, yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in the fruit mesocarp (Fruit a*), internal fruit breakdown (IBD) and penetrometer readings as an indication of mesocarp firmness (PEN). With the exception of CHL and LW, all characteristics displayed significant mean square differences for genotype x location x season interactions, suggesting differential ranking of genotypes across environments. Phenotypic variability attributed to genetic variation ranged from 14% to 33% in plant morphological characteristics, 16% to 62% in yield and yield-dependent traits and 50% to 67% in fruit quality characteristics, supporting the existence of immense inherent variability within the population. The additive main effect and multiplicative interaction analyses across six environments indicated internal fruit quality characteristics to be more stable across environments than AFM, FN and yield. None of the genotypes was stable for all characteristics. Based on stability and performance, G11 and G13 were identified as the most desirable genotypes for the processing and small-fruited market segments respectively. Similarly, both G16 and G17 were most desirable for the fresh market segment. Based on the genetic components estimated using 27 $F_1$ genotypes, AFM, FN, TSS, DMC, Fruit a* and PEN were observed to be under additive genetic control, implying selection in early generations would be effective for their improvement. In contrast, heterosis breeding could be more effective for the improvement of Leaf a*, DMY, uniformity and yield. Moderately high broad-sense heritability with lower genetic gain as a percentage of the mean was recorded for CHL, Leaf b*, LW, PL and IBD, suggesting additive genetic control although

the environment plays a larger role in the expression of the phenotypes. A strong negative association was observed between AFM and FN as well as a strong positive correlation between DMC and TSS. CHL had moderate correlations with AFM and FN. Weak negative correlations were also estimated between yield and internal fruit quality characteristics and more specifically TSS, DMC and Fruit a*. Findings from the line x tester analysis, involving four lines and four testers, showed none of the parents to be consistently good general combiners for all characteristics. Leaf a*, Leaf b*, FN, TSS, DMC and PEN revealed non-significant line x tester interaction mean squares. LW, PL, AFM, Fruit a* and IBD were found to be predominantly under additive genetic control. CHL, DMY, uniformity and yield were demonstrated to be mostly under non-additive genetic control. Significant mid-parent heterosis was estimated for all characteristics with heterosis percentages above 45% for AFM, DMY, FN and yield. Using the current germplasm collection, the most feasible strategy for the improvement of butternut genotypes would be through selection in early segregating generations. For yield, uniformity and DMY more desirable results will be achieved through heterosis breeding. Focussing on yield, FN and AFM, in combination with DMC and TSS, will be the most effective approach to develop high-performing, stable and desirable hybrids.

***Keywords*****:** Butternut, combining ability, correlation, genetic advance, genotype x environment interaction, heritability, heterosis, line x tester analysis, phenotypic diversity, stability

# CHAPTER 1

## INTRODUCTION

*Cucurbita moschata* is part of the *Cucurbita* genera, which was collectively part of the 10 leading vegetable crops worldwide in 2018 (FAO, 2018). In the same year, butternut ranked as the sixth most important vegetable in South Africa (DAFF, 2018). The majority of these productions consisted of varieties developed in foreign countries, which should leave room for improvement of material adapted specifically to South African conditions. Compared to other crops, the number of commercial butternut breeding programmes are limited, which makes butternut breeding attractive.

Authors from across the globe refer to *C. moschata* as one of the most important household vegetables, important in food security and grown under a wide variety of agro-climatic conditions (Akter *et al.,* 2013; Carvalho *et al.,* 2014; El-Tahawey *et al.,* 2015; Naik *et al.,* 2015; Darrudi *et al.,* 2018). Considerable diversity for fruit shape, size and colour are preserved in landraces with limited attempts made to exploit this variation in crop improvement programmes (Jahan *et al.,* 2012; Oliveira *et al*., 2016; Mohsin *et al.,* 2017).

In recent years, biofortification has been used to alleviate micronutrient malnutrition in developing countries affecting women, children and infants (Shafiin *et al*., 2020). Since butternut is an affordable source of carotenoids (Tamilselvi *et al*., 2012), the development and release of improved varieties can make a significant contribution to human livelihoods in poor countries. Butternut is not only an important source of nutrients but has good storability, superior transport potential and a long period of availability, all contributing to its increasing popularity (Hazra *et al.,* 2007).

For many years, Starke Ayres, a vegetable seed company in South Africa, focussed mainly on germplasm involving bell-shaped fruit, aiming to improve yield. As a result Starke Ayres butternuts have gained a lot of market share in recent years with the most recent released varieties comparing well with opposition material. However, in order to be market leaders, new hybrids need to be superior. Improvement of internal fruit quality will not only benefit vegetable processors, but could also make a significant contribution to the nutritional requirements of small-scale farmers in Africa.

Recently the Starke Ayres butternut germplasm was extended to include more variation in morpho-agronomic and internal fruit quality characteristics. More stable genotypes showing less

environmental variation were also added to the gene pool. In future, these characteristics will be a requirement for the release of new material.

The initial stages of any crop improvement programme include the understanding of genetic behaviour of desirable characteristics in formulating an appropriate breeding strategy. Although many studies included estimation of genetic parameters of various characteristics in *C. moschata*, a comprehensive study focussed on both morpho-agronomic and internal fruit quality characteristics, specifically in butternut, is still lacking. Further investigation is needed for the application of classical breeding for the improvement of internal butternut fruit quality without losing yield. A comprehensive study is required to quantify variation within the germplasm, specifically for those characteristics that previously showed limited variation, and to establish which characteristics have the greatest scope for improvement of butternut.

### 1.1 Aim and objectives

The general aim of this study was to develop an effective breeding strategy in a South African context to improve butternut fruit quality, without a significant reduction in yield.

The specific objectives were to:

i. Quantify the phenotypic variability in morpho-agronomic and internal fruit quality characteristics of Starke Ayres butternut squash germplasm.
ii. Confirm the combination of high performance and stability for both morpho-agronomic and internal fruit quality characteristics in desirable butternut genotypes across different environments.
iii. Estimate the genetic parameters in Starke Ayres butternut germplasm in order to establish selection feasibility as well as to evaluate potential correlations between 15 morpho-agronomic and internal fruit quality characteristics.
iv. Investigate the magnitude of general and specific combining ability variances to gain insight into the gene action involved in the control of the 15 morpho-agronomic and internal fruit quality characteristics studied.

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# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Brief background and history of *Cucurbita*

The *Cucurbitaceae* family consists of approximately 100 genera and 800 species depending on the type of classification. More traditional morphological classifications refer to less genera and more species, but more advanced genotypic classifications identified more genera and less species (Rahman, 2013; Renner and Schaefer, 2016; Grumet *et al.,* 2017; Rolnik and Olas, 2020).

*Cucurbitaceae* is best known for their large, colourful and morphologically variable fruit that are produced in widely diverse forms throughout the world, both in the tropics and warm temperate regions. This diversity extends to bitter or aromatic, sweet or bland, and huge differences in storability ranging from highly perishable to storable for months with little change in quality. The most economically important species include watermelon (*Citrullus lanatus* Thunb.), melon (*Cucumis melo* L.), cucumber (*Cucumis sativus* L.) as well as various pumpkins and squashes (*Cucurbita* L.) (Grumet *et al.,* 2017). In general, bitter gourd, bottle gourd, wax gourd, sponge gourd, ridge gourd and snake gourd are of minor importance and mostly produced by small-scale farmers in Asia (McCreight, 2016).

*Cucurbita* L. species show phenomenal variation in fruit morphology and include numerous globally and regionally important crops. The terms “pumpkin” and “squash” are indiscriminately used to refer to the different cultivated species of the genus *Cucurbita* L. viz.: *C. pepo* L., *C. maxima* Duch., *C. moschata* Duch., *C. argyrosperma* Huber and *C. ficifolia* Bouché (Ferriol and Pico, 2008). In addition to these five most important species, there are at least another 10 wild species in this genus (Sun *et al*., 2017; Lopez-Anido, 2021). The original meaning of the word “pumpkin” referred to an edible round or nearly round fruit while “squash” referred to an edible non-round fruit. Inedible *Cucurbita* fruit are referred to as gourds but fruit from other *Cucurbitaceae* genera can also be included (Paris and Brown, 2005).

There are further overlaps whereby immature fruit of any of these species are referred to as “summer squash” and mature fruit as “winter squash”, respectively. Summer squash consists of marrow, zucchini, scallop, yellow straightneck and yellow crookneck varieties, while winter squash includes butternut, buttercup, delicata, hubbard, kabocha, turban and spaghetti squash (Robinson and Decker-Walters, 1997).

Prior to subsequent distribution and diversification in Africa and South America, origins of *Cucurbitaceae* trace back to Southeast Asia but only a small percentage of this great diversity was domesticated to current crop status (Grumet *et al.,* 2017). Paris and Brown (2005) refer to various authors and suggested most of the wild *Cucurbita* species still grow in broadly scattered regions in Mexico. More recently, the taxa have been described to occur as widely as the midwestern United States to Central America as well as to areas as far as southern Argentina with the greatest diversity in central Mexico and western borderlands between the United States and Mexico. These areas show great ecogeographic variation regarding temperatures and precipitation (Khoury *et al.,* 2019; Lopez-Anido, 2021).

The five *Cucurbita* species mentioned earlier were selected by Native Americans long before the discovery of the Americas, with the domestication of *C. pepo* perhaps having begun 10 000 calibrated years before the present (Khoury *et al.,* 2019). Archaeological remains of *C. moschata* suggested cultivation in the Andes from the same era, making squashes one of the very earliest components of food production systems in South America (Watling *et al.*, 2018). Tropical pumpkins were grown in eastern and southwestern United States in pre-Columbian times. *Cucurbita* species were introduced to other continents by the turn of the 16$^{th}$ century and since then they became important in many areas outside of the Americas. The species spread globally, leading to great diversification across Europe, Asia and Africa. Based on early reports of European explorers, *C. moschata* was introduced to Europe as early as *C. pepo* but did not appear until much later and not as abundantly. In many developing countries *C. moschata* is still being cultivated extensively as landraces and used as a staple food (Akter *et al.,* 2013; Carvalho *et al.,* 2014; Naik *et al.,* 2015; Mohsin *et al.,* 2017a). Although numerous cultivars have been registered and referred to as day-neutral (Jahan *et al.,* 2012), the centre of diversity lies in the form of innumerable unnamed landraces in the American tropics. Many of these landraces cannot be cultivated at higher latitudes since they are adapted to short days but long growing seasons (Andres, 2004a).

The great diversity, and the absence of a known wild species closely related to *C. moschata*, left some uncertainty about the exact centre of origin for this species. Based on morphological and molecular analysis it is possible that two independent domestications happened in Central and South America all in the pre-Columbian era. An introgression from a related species could also be the reason for the separation between accessions from Central and South America (Andres, 2004b; Ferriol *et al.,* 2004). After the introduction to the Old World, secondary domestication centres in Asia resulted in great diversification (Sun *et al*., 2017). Nevertheless, it has been reported that *C. moschata* is closest related to *C. argyrosperma* and the second most diverse species in the genus after *C. pepo* (Lee *et al*., 2021).

## 2.2 Botanical description

Cucurbits are frost-sensitive and produce predominantly tendril-bearing vines (Robinson and Decker-Walters, 1997). Domesticated species were selected from mesophytic annuals having fibrous root systems. The herbaceous stems are long trailing vines with a prostrate growth and can reach up to 15 m in length. The vines produce large simple leaves, typically arranged alternatively one leaf per node (Lopez-Anido, 2021). The family is known to have medium-sized plants with inferior ovaries and parietal placentation, with their tendrils distinguishing them from their closest relatives. Although dioecy seems to be the ancestral condition, about 50% of the species are monoecious having both male and female flowers on the same plant. Pistillate flowers are distinguished by the ovary at the base of the petals with a thicker and shorter stalk than the staminate flowers (Hazra *et al.,* 2007). *Cucurbita* plants are monoecious, producing unisexual flowers and are therefore a cross-pollinating crop (Rolnik and Olas, 2020). Cucurbits have large showy orange-yellow, nectar-producing flowers, which attract pollinating insects. Domestic honeybees usually transport heavy, sticky pollen grains from staminate flowers to receptive stigmas of female flowers to result in fertilisation (Hazra *et al.,* 2007). These solitary flowers have a very short lifespan and open for a few hours only on a single day. The greatest variation among genotypes is expressed in the fruit (Paris and Brown, 2005). The fruit are botanically classified as fleshy berries, which vary morphologically with respect to size, shape and colour. Internal quality varies with regard to texture, colour and nutritional value. Some species are photoperiod sensitive while others are day-neutral (Kristkova *et al.,* 2004).

In South Africa, *Cucurbita moschata* is known as “butternut squash” but it is also popularly called pumpkin in Western countries (Hazra *et al.*, 2007). Cultivars adapted to temperate conditions tend to be moderate in size with non-lignified rinds. Fruit are usually uniformly buff-coloured or a reticulated dark green, smooth or moderately ribbed with orange flesh, light tan coloured seed and plants are grown mainly for their mature fruit. In other countries, fruit shapes resemble those of *C. pepo* with colours ranging from white, buff, yellow, orange and very dark green with various forms of mottling. Fruit shapes vary from flattened, globular, round to oblong and cylindrical as well as pear-, heart- and dumbbell-shaped. Surfaces can be warty and often ridged with sizes from small to more than 100 kg. Flesh colour also range from dark orange to pale yellow and in some cases blackish-green (Andres, 2004a, Ferriol *et al.,* 2004). In most *Cucurbita,* the stigma is yellow but the intense orange colour of *C. moschata* is a unique feature used for species determination. The *C. moschata* peduncle can also be used in identification, which is usually rigid, angular with obtuse ribs and enlarged at the attachment to the fruit (Kristkova *et al.,* 2004).

Cytogenetically, all *Cucurbita* species show uniformity with a diploid chromosome number of 2n = 40 and a relatively small genome size of 400 megabases (Grumet *et al.,* 2017). Despite vast phenotypic variation between species, none of the cultivated species within *Cucurbita* is reproductively isolated in terms of artificial hybridisation. *C. moschata* is widely cross-compatible and probably has the most ancestral-like genome. Interspecific crosses are widely used in commercial breeding programmes (Robinson and Decker-Walters, 1997).

**2.3 Economic importance of butternut**

*Cucurbita* species are grown mostly for their fruit, which are a significant source of carbohydrates and vitamins both for human consumption and animal feed, but can be used for ornamental purposes as well (Sojak *et al.,* 2014). Pumpkin and squash are widely used in the culinary world where they are enjoyed, cooked or pureed, and used as an ingredient in soups, stews, bread, pies and other dishes (Gomes *et al.*, 2020). Cucurbits can be stored for long periods but because it is a seasonal crop and grown in different regions, it is often processed by freezing or drying. Pumpkins are very versatile and can be used in both sweet and savoury food products. Although pumpkins can have high storability, they have a high moisture content, which makes them susceptible to deterioration. The high moisture content is the reason for their bulkiness and this hampers handling and transportation; therefore, production is promoted closer to consumption areas.

The most common form of preserving *C. maxima* in Nigeria is done by drying. The product can be stored for years without significant loss of nutrients (Falade and Shogaolu, 2010; Tunde-Akintunde and Ogunlakin, 2013). It has been reported that pumpkin shreds, granulated powder and fine powder as well as pumpkin seed powder are used as nutritional supplements in instant and ready to cook food mixes in India. Pumpkin powder has a long shelf life and is being used as a supplement in cereal flours for bakery products, sauces, instant noodles and instant pumpkin kofta. Butternut is also used as raw product for agro-industrial processing for the production of powder as a natural colouring agent for pasta and flour (Tamilselvi *et al.,* 2012; Durante *et al.,* 2014; Dhiman *et al.,* 2017). In Colombia, an integral biomass dry matter content of the fruit must be as least 20% for a genotype to be used in animal feed (Valdes-Restrepo *et al.,* 2013).

In recent years the consumption of pumpkin seeds, which are good sources of protein and vegetable oils, has increased significantly. Some of the variation in the elliptically flattened seed includes absence or presence of seed coat, and various colours including white, beige, tan orange, brown and black (Kristkova *et al.,* 2004). The oil is cholesterol-free and used for cooking, soap making and as domestic and industrial lubricants (Lawal, 2009). Some long-

fruited cultivars are primarily used for their immature fruit. Leaves and growing tips have been consumed as a vegetable in Africa and Mexico, while in Italy both male and female flowers are used in soups and other foods (Andres, 2004a; Lawal, 2009; McCreight, 2016).

Pumpkin and squash are grown in almost all arable regions of the world, from cool temperate to tropical. *C. pepo, C. maxima and C. moschata* have different climatic adaptations and, thus, distributed differently in global agricultural areas. Tropical pumpkins (*C. moschata*) are more prominent in tropical areas in less-developed countries, while zucchinis (*C. pepo*) are of high economic value in developed countries with temperate climates (Paris and Brown, 2005). *C. moschata* is one of the most important cucurbitaceous fruit grown due to its taste, nutritional value, storability, prolonged availability and transport potential (Hazra *et al.,* 2007; Jahan *et al.,* 2012; Rana *et al.,* 2015; Ahmed *et al.,* 2017; Kakamari and Jagadeesha, 2017).

In South America the use of interspecific crosses between *C. maxima* and *C. moschata* are commonly used for kabocha pumpkin production (Andres, 2004a). Various interspecific crosses are also used as rootstock for other *Cucurbitaceae* genera. *C. moschata* is the most important and wildly cultivated cucurbit in India, Africa, Latin America, southern Asia and the United States, but also the least studied (Hazra *et al.,* 2007; Naik and Prasad, 2016). This is not reflected in worldwide production statistics since it is produced commonly on a small-scale basis and locally consumed, with no record of production data (Andres, 2004b).

Yield varies greatly across continents and can be attributed to length of growing season, open field *vs.* protected cultivation, and management practices including irrigation, fertiliser and pest control. In areas of abundant rainfall, cucurbits are produced in the dry season but can also be produced in the driest deserts with external water sources. Raised beds are often used in field cultivation to improve drainage (McCreight, 2016).

Although *C. moschata* has been cultivated as winter squash since pre-colonial times, the typical butternut shape is a recent innovation. Crookneck fruit types were used in the United States until the origin of short-necked butternut fruit in the early 1930's (Mutschler and Pearson, 1987). Many other areas are still making use of flat, globular and oval-shaped fruit. For this reason, numerous research reports in this species do not include butternut type fruit shapes, and are not necessarily applicable to improvement of butternut type fruit varieties.

Global production figures do not report production area or yield among various types of squashes or pumpkins, but rather combine both summer and winter squash production, and both include fresh market and processing crops. The *Cucurbita* genera collectively formed

part of the 10 leading vegetable crops worldwide in 2018. China, India, Ukraine, Russian Federation and Mexico are the leaders in the world's production while South Africa ranked 19th (FAO, 2018). A total of 191 000 tons of *C. moschata* were sold to a value of R303 million in South Africa in 2018 (Figure 2.1). This is significantly higher than the combined 78 000 tons of *C. maxima* (pumpkin and hubbard squash) that equated to R186 million. These figures have been constant over the last 10 years and include data from the 19 largest fresh produce markets in South Africa, but exclude produce delivered directly to the major supermarkets and retailers (DAFF, 2018).

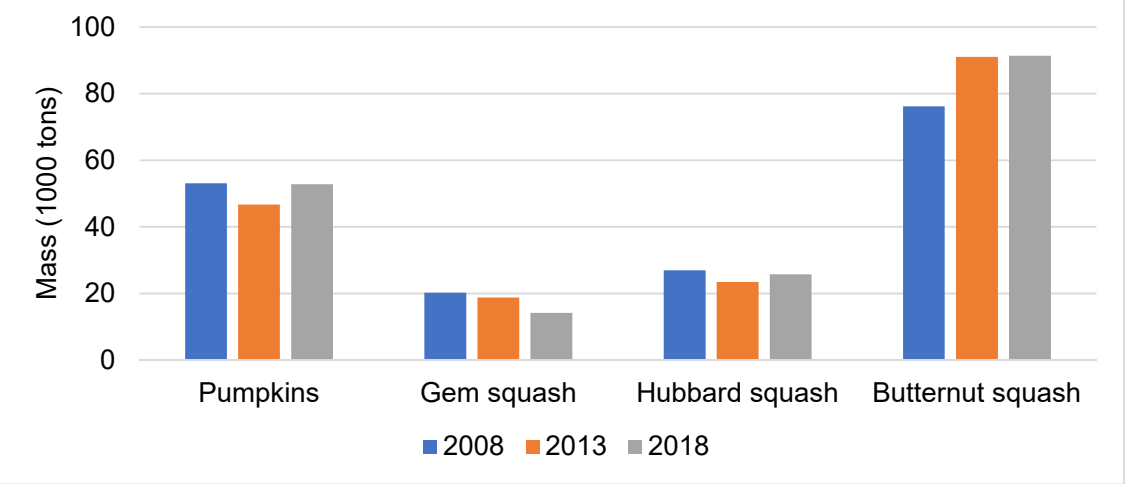


**Figure 2.1** Mass of mature cucurbit fruit sold on 19 South African fresh produce markets in 2008, 2013 and 2018. Source: DAFF (2018)

Cucurbits are subject to many insect pests and diseases, with powdery mildew and various viruses being the most important. Their control includes cultural practices, chemical practices and host plant resistance, all with varying levels of success. Using resistant rootstocks are becoming more important, especially where different genera can be graft-compatible (McCreight, 2016; Montero-Pau *et al.,* 2016). Due to tropical pumpkin's tolerance to poor tropical soils, their rootstocks are relatively successful under marginal conditions resulting in resistance to biotic and abiotic stresses, including soilborne diseases (Sun *et al*., 2017).

### 2.4 Important characteristics of butternut

Characteristics, which are useful in the breeding of butternut, have been described in literature. These include fruit size, both in terms of weight or fruit dimensions, and yield per plant. A comprehensive study, which quantifies variation for these characteristics and more specifically fruit quality characteristics, is required since they have received limited attention to date, especially across different environments.

Development of high-yielding genotypes requires information about the magnitude of variation in the available genotypes. The first step in any improvement programme should be to select high-yielding genotypes with desirable characteristics (Tamilselvi *et al.,* 2012). The importance of and variation within different butternut characteristics are, therefore, further discussed.

### 2.4.1 Leaf canopy characteristics

Canopy colour in *C. moschata* is influenced by various factors. The dominant contributing factor would be the green colour of the leaf blade, which varies from light to dark green and is genetically controlled (Du *et al.,* 2011; Kiramana and Isutsa, 2017). In addition, silver mottling is a heritable characteristic influencing differences in the distribution of silver-grey colouring over the leaf surface. This can vary from completely absent to a very high percentage of the leaf blade that is being covered (Ribeiro and Da Costa, 1989; Paris and Padley, 2014).

In order to evaluate colour objectively, various colour models are available. The most frequently used models include RGB (red, green, blue), *L*a*b** (luminance and intensity of the colour space values) and HSB (hue, saturation, brightness). In the RGB model, each parameter captures the intensity of the light in the red (R), green (G) or blue (B) spectrum respectively, with theoretical values ranging between 0 and 255 (Leon *et al.,* 2006). *L*a*b** is an international standard for colour measurement that correlates well with the perception of the human eye. *L** is the lightness component or luminance (L* = 0 for black, L* = 100 for white), *a** describes colour intensity in red (a* > 0) or green (a* < 0) and *b** describes colour intensity in yellow (b* > 0) and blue (b* < 0). Hue angles (H°) range from 0 to 360 where each degree represents a distinct colour (Seroczynska *et al.,* 2006).

In addition to physical characteristics such as colour, contemporary agricultural demands have inspired plant breeders globally to investigate improved physiological characteristics, which correlate with plant growth, development and yield. Therefore, analysis of photosynthesising material for chlorophyll fluorescence is an important approach to evaluate health and integrity of plant material. Photosynthesis is often reduced in plants experiencing unfavourable conditions such as water deficit, nutrient deficiencies, temperature extremes and pathogen attack. Measurement of chlorophyll content, using a hand-held chlorophyll meter is fast, non-invasive and non-destructive (Samsone *et al*., 2007), which allows large-scale screening of plant populations.

The close relationship between leaf chlorophyll content, photosynthesis efficiency and resistance to environmental stress suggests photosynthetic efficiency to be highly correlated

with yield and yield components, and it can be very useful in breeding programmes (Kalaji and Guo, 2008; Karademir *et al.,* 2009). In maize, high yielding varieties with high chlorophyll levels were, in more than half the cases, drought tolerant (Khayatnezhad *et al.,* 2011). Chlorophyll content is influenced by genotype, temperature, leaf age and several environmental factors, including water availability. Cultivars with resistance to these environmental factors had higher chlorophyll levels. It was also demonstrated that chlorophyll content could be used as an indication of drought tolerance in short growth period potato cultivars (Van der Mescht *et al.,* 1999) as well as in barley (Rong-hua *et al.,* 2006). Photosynthetic activity is not only genetically controlled in plants but is also under environmental influence. By selecting butternut genotypes, with higher chlorophyll content across different environments, yield might be improved (Ghimire *et al*., 2015). Unfortunately, chlorophyll-reading instruments are costly and have a small sampling area that is subject to operator bias.

### 2.4.2 Yield and yield components

Yield is a complex characteristic since it is not only under genotypic control but also largely influenced by the environment. For this reason, selection based on yield alone could be ineffective for the improvement of yield in *Cucurbita* (Sojak *et al.,* 2014). However, the great variation in various yield component characteristics among genotypes suggests good scope for improvement of economically important characteristics, including yield, through traditional selection techniques (Pandey *et al.,* 2008). In grain crops, it has been confirmed that selection for yield along with its components are more effective and reliable than selection for yield alone. Therefore, knowledge of the impact of various yield components could be essential for selection of desirable butternut genotypes (Tamilselvi *et al.,* 2012; Fasahat *et al.,* 2016).

Literature refers to various yield component characteristics related to *C. moschata*. Some of the morphological characteristics include length of the main vine, number of primary branches and leaf area (Nisha and Veeraragavathatham, 2014; El-Tahawey *et al.,* 2015; Hussein and Hamed, 2015; Kakamari and Jagadeesha, 2017). Due to a possible relationship between flowering date and fruit maturity, various authors associate flowering characteristics, such as number of male and female flowers per plant and the first node at which the first male and female flowers are developing, with earliness (Jha *et al.,* 2009; Tamilselvi *et al.,* 2015; Ahmed *et al.,* 2017; Mohsin *et al.,* 2017b). Similarly, number of days to first harvest was used as an indicator for earliness, which could be linked to premium prices where growers were able to catch the early market (Tamilselvi *et al.,* 2012).

Along with the above, additional studies indicated that average fruit weight, shape, length, diameter and number are all yield components (Pandey *et al.*, 2008; Naik and Prasad, 2016;

Abdein *et al.*, 2017). Although strong associations between average fruit weight and fruit dimensions have been reported, these relationships were also influenced by internal fruit quality (Rana *et al.,* 2015; Restrepo-Salazar *et al.,* 2019). Number of fruit produced is usually a good indicator of yield in any vegetable where a higher fruit number will ultimately result in higher yield (Tamilselvi *et al.,* 2012). Although certain regions still prefer larger fruit, preference has recently changed towards small to medium-sized fruit (Tamilselvi *et al.,* 2012). There are also different market segments within the same regions, where larger fruit sizes are used for the processing sector and smaller fruit preferred for fresh market consumption.

In pumpkins with a flat to globular fruit shape, thick fruit flesh around the seed cavity is a desirable quality trait (Tamilselvi *et al.,* 2012). However, in butternut fruit types, neck length and neck thickness are of greater importance than the flesh thickness around the seed cavity. Although genotypes are fairly uniform in their phenotypic expression, since these are maintained through inbreeding, genotypes still show a relatively high amount of variation in fruit shape and size, specifically due to environmental influences.

Various authors referred to flesh thickness as an indication of the relative size of the seed cavity compared to the fruit size. This is beneficial where the fruit flesh will be consumed (Hussein and Hamed, 2015; Rana *et al.,* 2015; Abdein *et al.,* 2017; Ahmed *et al.,* 2017). However, a larger seed cavity can also be beneficial in pumpkins where seed production for consumption is the main objective (Nisha and Veeraragavathatham, 2014; Mohsin *et al.,* 2017b; Darrudi *et al.,* 2018; Restrepo-Salazar *et al.,* 2019). Although thicker flesh may result in higher yield, flesh quality is of utmost importance. Unfortunately, it was demonstrated that higher yield could be linked to poorer flesh quality (Akter *et al.,* 2013).

### 2.4.3 Internal fruit quality characteristics

Flesh quality in *Cucurbita* is a combination of colour, texture, nutritional value and flavour, and is dependent on various metabolic pathways (Wyatt *et al.,* 2014). Differences are influenced by genetic differences, growing conditions, maturity and post-harvest-storage handling (Murkovic *et al.,* 2002; Azevedo-Meleiro and Rodrigues-Amaya, 2007; Kimura *et al.,* 2007; Zaccari and Galietta, 2015). Growing conditions alone show great variation and are determined by temperature, nutrient and water availability, soil type, light intensity, season and climate (Schmidt *et al.,* 2005; Sojak *et al.,* 2014).

In *Cucurbita*, mature fruit size is reached around 20 to 25 days after pollination but maturity is only reached 55 to 60 days after fruit set (with the completion of seed fill). Harvesting prior to maturity requires remobilisation of metabolites from the flesh during storage, which can reduce

flesh quality substantially. In addition, mature fruit can also be stored before consumption. Post-harvest storage of fruit and vegetables is often required and frequently results in a change in nutritional quality. The period as well as the post-harvest conditions play an integral part in internal fruit quality (Schmidt *et al.,* 2005; Sojak *et al.,* 2014). In *Cucurbita* this “curing” is generally accepted as improving quality due to conversion of starch into sugar, and the continuous increase of carotenoids during the first two months of storage (Zhang *et al.,* 2014).

After carrots, the cheapest source of carotenoids is pumpkin. For this reason total carotenoid content is a nutritionally important parameter to consider (Tamilselvi *et al*., 2012). *C. moschata* is an affordable source of carotenoids and is an important component in the daily diet as vegetable (Rodrigues-Amaya and Kimura, 2004; Faber *et al.*, 2013), but limited effort has been put into the development of improved high yielding varieties rich in carotenoids.

Preformed vitamin A and provitamin A carotenoids are the two forms of vitamin A (retinol) available in the human diet. Preformed vitamin A can be found in animal-derived foods like meat, fish and dairy products. Carotenoids are plant pigments, which the human body convert to vitamin A. The most important provitamin A carotenoid is β-carotene. In the United States, the recommended daily allowance of vitamin A for a healthy adult is 900 µg for men and 700 µg for women (NIH, 2019). The recommended dietary intake for German-speaking countries is 1 mg per day and 2.5 mg to 5 mg per day in Poland, which is equivalent to 15 mg to 30 mg β-carotene per day (Sojak *et al.,* 2014).

A study of 25 *C. moschata* genotypes showed large within species variability in carotenoid content with total carotenoids varying in fresh pumpkin from 124.6 $\mu g.g^{-1}$ to 699.06 $\mu g.g^{-1}$ (Carvalho *et al.,* 2015). Similar results were obtained where total carotenoid, after 60 days of storage, varied from 274 $\mu g.g^{-1}$ to 623 $\mu g.g^{-1}$ and 82-239 $\mu g.g^{-1}$ for *C. maxima* and *C. moschata* respectively (Bonina-Noseworthy *et al.,* 2016).

Similar to carotene accumulation, both total soluble sugars (TSS) and dry matter content (DMC) remain relatively low during early fruit development but show a dramatic increase 40 days after pollination. There are also post-harvest similarities where carotenoids and TSS increase gradually during post-harvest storage up to 45 days when the maximum is reached, followed by a decrease as time progresses (Rahman *et al.,* 2013). In another study, glucose increased sharply in the second month of storage with starch metabolised to soluble sugars, which are used during respiration for maintenance of fruit after harvest. Following the sharp increase, glucose decreased at four and six months to levels lower than original levels at

harvest (Zaccari *et al.,* 2017). It was indicated that the genes involved in biosynthesis of starch, sugar and carotene levels in squash correspond with those in other plants (Wyatt *et al.,* 2014).

Starch breakdown during storage is associated with amylochromoplasts, which harbour both starch and carotenoids and that progressively transform into chromoplasts, containing mainly carotenoids (Zhang *et al.,* 2014). The production of higher-level compounds such as sugar, starch and carotenoids are highly correlated with consumer acceptance. Sweetness is positively correlated with flavour, while starch content correlated with texture and influences DMC (Wyatt *et al.,* 2014).

Both DMC and TSS of the fruit mesocarp are major attributes for determining acceptable eating quality. DMC in *C. maxima* ranged from 25% to 37% with a mean of 32% at harvest but decreased to 24% and 22% after storage for 30 and 60 days respectively. The DMC in *C. moschata* cultigens was considerably lower with an average of 22% at harvest, and 18% and 19% at 30 days and 60 days respectively after harvest (Noseworthy and Loy, 2008).

Although cultural practices are similar for the various winter squash species, harvesting schedules and post-harvest handling may vary significantly across production areas, resulting in different eating qualities and nutritional values. Furthermore, nations differ in their preference for the degree of moisture in squash and flavour components. According to Maynard *et al.* (2002), areas in the Caribbean prefer fairly moist squash with a DMC of between 10% and 15%, while Asian countries prefer a much higher DMC (20% to 33%) in order to qualify as excellent eating quality. Butternut varieties used in North America have a DMC range of 15% to 21% (Noseworthy and Loy, 2008). In South Korea, DMC is about 10% (Seo *et al.,* 2005). It was mentioned that a TSS content of 11 °Brix seemed to be acceptable for good eating quality in *C. maxima* but DMC above 28% to 30% in squash is probably too dry for normal consumption, even if TSS levels are at 11 °Brix. Since *C. moschata* has a lower DMC, slightly lower TSS levels should still be acceptable in terms of good eating quality (Noseworthy and Loy, 2008).

**2.5 Less important characteristics**

Numerous studies and multivariate analyses have confirmed genetic diversity in *C. moschata* (Du *et al.*, 2011; Grisales *et al.*, 2015; Naik *et al.,* 2015; Tamilselvi *et al.,* 2015; Mohsin *et al.*, 2017b). Most studied traits confirming genetic dissimilarity include days to first male and female flowers, number of first male and female flower nodes, vine length and number of primary branches. Many of these traits have been studied using small numbers of plants, planted using a wide spacing (3 m x 3 m). Many studies also refer to seed traits, which are of

limited value if the objective is not seed yield for seed consumption. Due to the trailing nature of the species, plants tend to grow into one another during flowering. Therefore, information regarding these characteristics is of limited use in a commercial breeding programme where higher plant densities are typical, making it impossible to evaluate these characteristics.

### 2.6 Breeding considerations in *Cucurbita*

Cucurbits have been greatly improved over the past century using conventional plant breeding methods. Aside from more efforts recently, plant breeders focused mainly on increasing productivity, yield, fruit size and quality. The current focus is on incorporation of resistance to diseases, in particular virus resistance. This can be accomplished by using germplasm collections from around the world, and with molecular breeding approaches, which play an increasingly important role. Most of these traits are under monogenic control (Grumet *et al.,* 2017; Khoury *et al.,* 2019).

*Cucurbita* species are highly polymorphic, stimulating much research on inheritance of fruit characteristics (Paris and Brown, 2005). Parts of the vast phenotypic diversity in *Cucurbita* have been studied and published in various gene lists. The most recent gene list was published in 2014 and various qualitative characteristics were included. These include traits referring to plant architecture (bush growing habit, leaf and tendril morphology and disease resistance traits) as well as colour inheritance (stem colour, rind colour and flesh colour). Great increases in plant productivity have been accomplished through changes in plant architecture. Similarly, in *Cucurbita,* compact growing habits have been incorporated, particularly in *C. pepo* summer squash varieties. In the past 40 years, great improvements have been made in disease resistance breeding but mostly in summer squash varieties (Andres, 2004b; Paris, 2017). In recent years, great advances have been made in the development of hybrid cultivars with fruit traits as per specific requirements, uniformity, earliness and high marketable yields (Dhillon *et al*., 2020). Although a number of studies have been performed in Asia and South America on flat- and round-shaped fruit, none to very little information is available on bell-shaped butternut types.

Although there are still open-pollinated varieties available, there is a huge demand for the development of hybrid cultivars. Fruit size has been improved during domestication but crop productivity has received little attention given the importance of *Cucurbita* as a food source (Ferriol and Pico, 2008). Although more emphasis has been placed on molecular breeding approaches, not much has changed over the last 20 years where breeding objectives for winter squash varieties still include increased productivity, improved morphology, darker internal fruit colour and resistance to pests and diseases (Dhillon *et al*., 2020).

### 2.6.1 Heterosis

A monoecious species like *C. moschata* has a virtually obligatory outcrossing system and is, therefore, more prone to heterosis (Jahan *et al.,* 2012) but cucurbits do not necessarily show significant loss in vigour due to inbreeding. The lack of inbreeding depression in butternut does not necessarily imply the absence of heterosis (Hazra *et al.,* 2007; Acquaah, 2012). Inbreeding is widely used to attain uniformity, improve yield by individual plant selection and to recombine valuable characteristics from different inbred lines in hybrids. However, selection on its own as a form of inbreeding might not necessarily lead to remarkable results in all characteristics (Nisha and Veeraragavathatham, 2014). In addition, the huge genetic diversity available in *Cucurbita* paves the way for the development of hybrids through the exploitation of heterosis breeding (Tamilselvi *et al.,* 2015).

Heterosis is attributed to heterozygosity due to superior genes contributed by both parents (Nisha and Veeraragavathatham, 2014). Heterosis represents the superiority in performance of hybrid individuals compared with their parents, and is usually expressed as mid-parent or better-parent heterosis (heterobeltiosis) (Restrepo *et al.,* 2018). In a Colombian study, six open-pollinated varieties ($S_0$) were selfed to produce $S_1$ and $S_2$ generations. The results demonstrated that a greater level of heterosis could be expected from material with a higher degree of inbreeding. Mean heterosis for yield and average fruit weight were recorded as 113% and 78% respectively for hybrids between $S_2$ generations compared to 59% and 43% respectively for $S_1$ generations (Restrepo *et al.,* 2018).

Numerous studies have confirmed the advantages of $F_1$ hybrids over open-pollinated varieties. Plant morphological characteristics exhibited extreme heterosis of 325% for number of female flowers (Jha *et al.,* 2009), 82% for main stem length, 48% for leaf area (El-Tahawey, *et al.,* 2015) and 67% for number of branches (Hussein and Hamed, 2015). Negative heterosis, as an improvement in earliness, has also been recorded at -18% for days to first female flowers and -39% for first female flower node (Tamilselvi *et al.,* 2015). Heterosis was also recorded for various yield component characteristics, which included 171% for yield per plant (Ahmed *et al.,* 2017), 203% for average fruit weight (Darrudi *et al.,* 2018), 42% for flesh thickness (Nisha and Veeraragavathatham, 2014) and 113% for number of fruit per plant (Jha *et al.,* 2009). Internal fruit quality characteristics also demonstrated heterosis of 120% for total carotenoids (Pandey *et al.,* 2010) and 71% for total carbohydrates (Tamilselvi *et al.,* 2015).

Although heterosis has been measured for various characteristics in *C. moschata,* limited information is available. Additional heterosis investigations in butternut are needed to identify which characteristics could be improved through hybrid breeding. If different yield components

can be improved through heterosis, the cumulative or synergistic effects of heterosis for yield components could improve yield significantly (Ahmed *et al.,* 2017).

### 2.6.2 Combining ability

The performance of a parent *per se* does not necessarily reveal its potential in combination with other parents, due to complex gene actions. Combining ability is an estimation of the value of a specific genotype based on the performance of its progeny. General combining ability (GCA) and specific combining ability (SCA) are two separate concepts used in hybrid breeding. General combining ability is the average performance of a genotype in a series of hybrid combinations and is a main effect largely due to additive gene action. A high GCA is associated with higher heritability with less influence from the environment. In these cases, higher achievement through selection is possible and characteristics can therefore be reliably fixed (Nduwumuremyi *et al.,* 2013). In the absence of non-additive gene action, SCA mean squares are not significant, suggesting the best performing hybrid can be produced by crossing the parents with the highest GCA (Baker, 1978; Tang and Xiao, 2013).

Specific combining ability is the deviation from the performance of a certain hybrid combination based on the average performance of the parental lines due to non-additive effects. These effects are made up from dominance variance and epistatic interaction components, which include additive x dominance and dominance x dominance interactions. High SCA effects can manifest from crosses including good x poor general combiners where favourable additive effects from the good general combiner interact with the epistatic effects of the poor general combiner. High SCA can also originate from low x low general combiners due to dominance x dominance interactions, resulting in overdominance. Since overdominance is subjected to heterozygosity, this is non-fixable (Rana *et al.,* 2015; Mohsin *et al.,* 2017a).

Gene action involved in the expression of a trait is determined by the ratio between the combining ability variance components. Higher GCA variance ($\sigma^2_{gca}$) compared to SCA variance ($\sigma^2_{sca}$) is an indication of additive gene action. These characteristics should be improved through recurrent selection in early generations to exploit the genetic variation. GCA effects controlled genetically, which is heritable and transmitted to the offspring, explain this. Under these circumstances broad-sense heritability is expected to be close to narrow-sense heritability, with non-significant $\sigma^2_{sca}$ (Jha *et al.,* 2009; Tang and Xiao, 2013; Hussein and Hamed, 2015; Shakeel *et al.,* 2016).

The prevalence of non-additive genetic control becomes more prominent when the ratio ($\sigma^2_{gca}/\sigma^2_{sca}$) is less than unity (smaller than one). This scenario, in combination with high broad-sense heritability relative to narrow-sense heritability, indicates the presence of high dominance variance ($\sigma^2_D$). When $\sigma^2_D$ is larger than additive variance ($\sigma^2_A$), the characteristic could be improved through reciprocal recurrent selection, progeny selection (Kakamari and Jagadeesha, 2017) and later generation selection (Shakeel *et al.,* 2016). In these scenarios heterosis breeding has the largest scope for the improvement of these characteristics. This is an indication of the presence of both additive and non-additive gene action, with significant $\sigma^2_{sca}$ (Fellahi *et al.,* 2013). In grain crops, it has been confirmed that selection for yield, along with its components is more effective and reliable than selection for yield alone. This is an indication of the involvement of both additive and non-additive gene action in yield. Combining ability can also be used to group genotypes into different heterotic groups (Fasahat *et al.,* 2016).

Combining ability has been widely adopted in breeding programmes in order to compare performance of parental lines in hybrid combination, and to predict optimal genotype combinations for different traits in hybrids. It also provides valuable genetic information on the inheritance of a trait (El-Tahawey *et al.,* 2015). The exploitation of heterosis in these hybrids can lead to commercialisation or to accumulate fixable genes in improved material (Nisha and Veeraragavathatham, 2014; Ahmed *et al.,* 2017; Mohsin *et al.,* 2017a). These hybrids maximise variance in breeding populations in order to recognise superior transgressive segregants in segregating populations (Fasahat *et al.,* 2016). Combining parental lines into hybrids is also the quickest way of combining valuable characteristics into one genotype (Jha *et al.,* 2009; Hussein and Hamed, 2015).

Various diallel studies have been conducted in *Cucurbita*. All studies estimated both GCA and SCA to be significant for most, if not all, traits studied. These studies showed significant SCA effects for almost all crosses, but none of the crosses were significant for all the traits. In addition, in these studies the mean squares for GCA was compared to the mean squares for SCA (GCA:SCA) in order to estimate the type of gene action involved for specific traits. Vine length consistently had a ratio larger than unity, which suggested the trait to be under additive genetic control, and that improvement is possible through recurrent selection (Nisha and Veeraragavathatham, 2014; El-Tahawey *et al.,* 2015; Hussein and Hamed, 2015). The number of primary branches, days to first male and female flowers, first male and female node, and number of male and female flowers showed dramatic variation among studies, suggesting both additive and non-additive gene action (Jha *et al.,* 2009; Tamilselvi *et al.,* 2015; Ahmed *et*

*al.,* 2017; Kakamari and Jagadeesha, 2017; Mohsin *et al.,* 2017a). Except for El-Tahawey *et al.* (2015) who found leaf area to be predominantly under additive control, no reference was made in any of these studies to specific leaf or plant architectural characteristics. In a maize study, it was demonstrated that both GCA and SCA effects were significant for chlorophyll content but the GCA effects were of greater importance (Betran *et al.,* 2003).

A number of *Cucurbita* studies on yield and its components offer contradicting results. Rana *et al.* (2015), Tamilselvi *et al.* (2015) and Kakamari and Jagadeesha (2017) illustrated that most yield components are under both additive and non-additive genetic control whereas Nisha and Veeraragavathatham (2014), El-Tahawey *et al.* (2015), Hussein and Hamed (2015), Ahmed *et al.* (2017) and Mohsin *et al.* (2017a) found yield and its components to be mostly under additive control. Jha *et al.* (2009), Pandey *et al.* (2010) and Abdein *et al.* (2017) found yield to be under additive control but various yield components under both additive and non-additive control.

Limited information is available on fruit flesh quality characteristics with regard to genetic control in *C. moschata.* Both DMC and β-carotene content appear to be under additive control (Rana *et al.,* 2015). Pandey *et al.* (2010) showed that total carotene was under additive control but Tamilselvi *et al.* (2015) showed the involvement of non-additive gene action as well. Abdein *et al.* (2017), Ahmed *et al*. (2017) and Mohsin *et al.* (2017a) demonstrated TSS to be under additive control but Rana *et al.* (2015) also found significant non-additive gene action.

Selection of parents is easy when a character is under unidirectional control by a set of alleles and additive effects are prominent. In these cases, evaluation based on *per se* performance and combining ability effects would be parallel and can be explained by the selection of parents with a good reservoir of superior genes. However, when non-additive gene effects are involved this would not hold promise in producing superior hybrids. In most cases, parents with high mean performance have significant GCA effects. Under certain conditions non-additive gene action may be triggered, resulting in high SCA effects and mean values of the responding hybrid, which may be due to dominance, epistatic and environmental influences. It would be more appropriate to evaluate hybrids based on all three criteria namely *per se* performance, SCA and heterosis (Tamilselvi *et al.,* 2015).

There is no consensus in literature about the type of gene action predominating the expression and genetic control of yield and its components or fruit quality traits in *Cucurbita*. However, most studies estimated highly significant variances for both GCA and SCA for most of the characteristics, if not all. These findings indicate that parents and crosses in different

populations differ significantly with regard to their GCA and SCA (Jha *et al.,* 2009; Hussein and Hamed, 2015). None of the parents were found to be a good combiner for all the traits, including yield, yield components and fruit traits. Frequently, good combiners crossed with one another do not necessarily result in good specific crosses, but superiority of poor combiners with poor combiners suggests overdominance and epistatic gene action. It is clear that both additive and non-additive characteristics play an important role in *C. moschata* breeding.

In literature, no reports on the comparison between combining ability rankings across seasons or environments in cucurbits could be found. Fasahat *et al.* (2016) referred to sorghum, linseed, cotton and maize where interactions between the environment and GCA were found. In these cases, with the exception of cotton, rankings for SCA changed across different environments. This is an indication that selection based on performance across environments would, perhaps, be more effective.

Combining ability estimates are influenced by the set of germplasm used as well as the testing environment and are, therefore, not a function of the specific genotype alone. This sheds some light on the contradictions in literature but still does not give clarity on specific gene actions involved in specific characteristics. Thus, further investigation in combining ability with specific focus on butternut material under South African conditions is necessary. In addition, parents with high GCA that can be used to improve the core Starke Ayres germplasm collection need to be identified. High SCA and high positive heterosis will indicate which characteristics are under the influence of non-additive gene action; therefore, it can be improved through hybrid breeding.

### 2.6.3 Line x tester analysis

Different mating designs are used by breeders to provide information on the genetics of specific traits (Awata *et al.,* 2018). The line x tester mating design is the most widely used in hybrid development and was introduced by Kempthorne in 1957 as mentioned by Muthoni and Shimelis (2020). Testers are usually easy to use, maximise expected yield in the crosses, reveal maximum information about the lines used in the crosses, have positive combining ability and acceptable *per se* performances. This design generates both full-sib and half-sib populations simultaneously, to reveal the best parents for use in a breeding programme and provide knowledge of the mechanisms that control the main agronomic characteristics for the improvement of the species (Fasahat *et al.,* 2016). The benefit of the line x tester mating design is its simplicity while still estimating SCA of each cross as well as GCA for both testers and lines (Nduwumuremyi *et al.,* 2013).

All cucurbit studies mentioned in the “combining ability” section were based on diallel mating designs, except for one line x tester analysis conducted on yield components and fruit quality, where all characteristics were under both additive and non-additive genetic control (Tamilselvi *et al.* (2015). To estimate combining ability for the identification of superior parental line combinations, multi-environmental trials, followed by statistical analysis, are needed. These can be used to separate genetic and environmental influences of which the genetic influences can be further partitioned into additive and non-additive components (Fasahat *et al.,* 2016). This will offer clarity on which characteristics ought to be focused on in early generations compared to advanced generations with emphasis on hybrid breeding.

### 2.6.4 Heritability

The phenotypic variation for a trait can be partitioned into genetic and environmental variation. Genetic improvement of both qualitative and quantitative characteristics of any crop depends on the amount of genetic variation available in the base population. The inherited proportion of variation observed in the progeny is known as the heritability of a trait. Heritability indicates the effectiveness with which selection of genotypes can be based on phenotypic performance (Acquaah, 2012), but provides no indication on the amount of genetic progress that would result from selecting the best individuals. The greater the genetic variability, the greater the scope for improvement through selection (Naik and Prasad, 2016).

Since different highly heritable characteristics can have different levels of variation within the same population, it makes sense to compare variation among traits in order to focus on those exhibiting higher levels of variation. The variance components in relation to the grand mean are referred to as the phenotypic coefficient of variation (PCV), genotypic coefficient of variation (GCV) and environmental coefficient of variation (ECV) and are useful in order to compare variation among traits relative to one another (Aruah *et al.,* 2012). The greater the variability for desirable traits within a population, the greater the chance of improving the crop for specific characteristics (Kumar *et al.,* 2011).

High PCV is an indication of a considerable amount of variation available, with a wide scope for improving the characteristic through selection. A high GCV indicates the existence of exploitable genetic variability of the trait through crosses, followed by selection in the segregating populations. A narrow range between PCV and GCV indicate that these characteristics are mostly governed by genetic factors with minimal influence of the environment on the phenotypic expression of the trait, indicating that selection based on phenotypic value would be effective (Aruah *et al.,* 2012; Jahan *et al.,* 2012).

Heritability can be calculated as the ratio of genetically-caused variation to the total variation, which includes the environmental variation. Estimates of heritability in *C. moschata* indicate a large amount of variation. This is to be expected since heritability is a property of not only the trait but of the population and environment as well. Any variation in these factors will result in a different estimate of heritability (Acquaah, 2012; Tang and Xiao, 2013).

Various authors studied different *C. moschata* landraces, selections and breeding lines and were in agreement with regard to the high level of variation in morphological characteristics, yield components and internal fruit quality characteristics. Harvested fruit weight per plant had in most cases the highest GCV compared to the other traits evaluated. Additional yield component traits, which also expressed high GCV values, were fruit length (Mohsin *et al.,* 2017b), number of fruits per plant (Kumar *et al.,* 2011; Jahan *et al.,* 2012), fruit equatorial circumference (Pandey *et al.,* 2008), number of seeds per fruit (Aruah *et al.,* 2012) and fruit and plant weight (Nagar *et al.,* 2017). These traits were coupled with high heritability that result in high genetic advances. This further indicates that they were less influenced by the environment and, thereby, confirm additive gene action. Selection for these characteristics would be beneficial for the improvement of yield (Mohsin *et al.,* 2017b).

Limited information is available regarding coefficients of variability involving fruit flesh quality. In an Indian study (Pandey *et al.,* 2003) involving *C. moschata*, both PCV and GCV were estimated to be high for ascorbic acid and β-carotene but the differences between PCV and GCV were relatively small. This indicated that the environmental effect was low, suggesting these characteristics could be easily improved through breeding. In a separate study yield, fruit dimensions and TSS were also estimated to have a high heritability with minimal differences between PCV and GCV, also suggesting minimal environmental influences (Mohsin *et al.,* 2017b).

In a similar study on *C. moschata*, characteristics with both high and low PCV and GCV showed high heritability. Therefore, genetic gain expressed as a percentage of the population mean were used to demonstrate more variation between traits. Traits with high heritability, PCV and GCV, and that showed high genetic gain, included mature fruit weight, plant weight and fruit yield per hectare and they were under additive genetic control. Highly heritable characteristics with moderate and low genetic gain were controlled by non-additive gene effects and selection for these traits (involving mostly characteristics influencing flower maturity) would be less effective (Nagar *et al.,* 2017).

High GCV and high heritability, coupled with high genetic advance as a percentage of the mean, were observed for various yield component and flesh quality characteristics, indicating that these are under additive genetic control and selection for improvement of these traits would be effective (Akter *et al.,* 2013). Similar results were obtained in other studies, where yield components and flesh quality traits showed high heritability and genetic advance, which suggested the preponderance of additive effects. However, yield itself showed lower genetic gain, indicating the involvement of non-additive gene action (Pandey *et al.,* 2008; Kumar *et al.,* 2011). This confirmed that fruit characteristics could be improved through selection while yield can be improved through hybridisation and heterosis breeding (Pandey *et al.,* 2010).

Limited information is available on the inheritance of flesh colour. Broad-sense heritability for *L*a*b** (luminance and intensity) colour space values in *C. moschata* and *C. pepo* ranged from 0.81 to 0.93 (Itle and Kabelka, 2009). It was suggested that effectiveness of genetic improvement through selection would be moderate to high. In the same study, heritability for α- and β-carotene, and total carotenoid content were calculated to be 0.85, 0.74 and 0.59, respectively.

Authors from across the globe including Zambia (Gwanama *et al.,* 2001), India (Pandey *et al.,* 2003; Naik *et al.,* 2015), Bangladesh (Akter *et al.,* 2013; Mohsin *et al.,* 2017a), Brazil (Carvalho *et al.,* 2014), Egypt (El-Tahawey *et al.,* 2015) and Iran (Darrudi *et al.,* 2018) refer to *C. moschata* in a very similar way. It is known as one of the most important household vegetables, playing an important role in food security and is grown under a wide variety of agro-climatic conditions. The species include a vast variation in local landraces with specific selection for shape, size and colour. Most authors were in agreement that, although a huge amount of variation is available to be exploited in breeding programmes, very little attempts have been made for genetic improvement of this crop (Jahan *et al.,* 2012; Mohsin *et al.,* 2017a). Further investigation is therefore needed regarding the range of differences between phenotypic and genotypic variances, which will give an indication of the environmental influence on morpho-agronomic and specifically internal quality characteristics in butternut. This will be used to estimate heritability as a function of expected genetic gain.

### 2.7 Genotype x environment interaction and stability

The species *C. moschata* is highly diverse, with market classification based exclusively on fruit morphology. Fruit shapes and sizes are highly variable with various rind and flesh colours. In addition, butternut is very sensitive to environmental variation, resulting in phenotypic variation within a plant for both fruit shape and size.

### 2.7.1 Genotype x environment interaction

Various quantitative characteristics are influenced by the environment, resulting in unpredictable performances across various growing environments. This phenomenon is known as genotype x environment (G x E) interaction and may result in low correlations between phenotypic and genotypic values, thereby reducing selection efficiency (Dia *et al.,* 2016). To overcome this problem genotypes are evaluated across multiple environments (locations and years) to select for stability and adaptability. This enables the breeding of improved crop varieties with stable and consistent performance across different environments and seasons (Osei *et al.,* 2018).

G x E interaction is commonly represented as the slope of the line when cultivar performance is plotted against an environmental gradient. Non-intersecting lines indicate that the rank of cultivar performance stays the same across environments. Intersecting lines represent a change in rank of cultivars across environments, implying optimum cultivars to be location specific (Zakir, 2018). Multi-location trials can be used to estimate G x E interactions. The benefits of multi-location trials are that limited experimental data can be used for accurate estimation and prediction of yield performance and stability. Genotype responses across environments can be determined that provide reliable guidance for selection of the most suitable genotypes for planting in new areas or in future years (Osei *et al.,* 2018). When the G x E interaction variance is found to be significant, various methods can be used to identify the most stable genotype(s). Currently, the additive main effects and multiplicative interaction (AMMI) model, and genotype main effect plus genotype x environment interaction (GGE) biplot methodology are the two most powerful tools used for the analysis of multi-location trial data (Osei *et al.,* 2018).

A combined analysis of variance, from a study on Columbian *C. moschata*, showed highly significant differences between genotypes and locations as well as for genotype by location interactions. Based on the line of the regressions, production per plant indicated to be unstable across different environments, whereas DMC was stable. DMC was, therefore, more predictable for both favourable and unfavourable environments. It was also concluded that yield as a production trait is genetically more complex compared to fruit DMC (Valdes-Restrepo *et al.,* 2013). In a report by Abdein *et al.* (2017), various yield component traits were studied, but seed weight per fruit was the only characteristic showing significant G x E interaction.

### 2.7.2 Additive main effects and multiplicative interaction

The AMMI model is a hybrid statistical model incorporating both analysis of variance (ANOVA) for the additive component and principal component analysis (PCA) for the multiplicative component, for analysing two-way (G x E interaction) data structures (Zakir, 2018). An attractive property of AMMI analysis is the graphically representation of genotypic and environmental data that helps to interpret the G x E interactions. Similar genotypes are clustered closer to each other than genotypes that are less similar. The same applies to environments (Malosetti *et al*., 2013). AMMI analysis is also used to identify the most stable accessions (Farshadfar *et al.*, 2011).

### 2.7.3 Genotype main effect plus genotype x environment interaction biplot

Stability analysis reveals those characteristics that are less influenced by the environment and that can be exploited through breeding. Differential ranking of genotypes can identify specific interactions between genotypes and environments across the environments. These interactions are used to identify genotypes with high general and specific adaptability. High general adaptability generally results in stable performance across a range of different environments.

Similar to the AMMI analysis, the GGE biplot method presents genotype performance and G x E interaction patterns graphically. Different views of the biplots are used to identify mega-environments and their associated most stable genotypes. Genotypes can also be ranked according to performance and stability, thereby identifying the most desirable genotype for each characteristic (Yan *et al.*, 2007). Although AMMI and GGE biplot analyses have been widely used in various grain crops and to a lesser extent in other vegetables, studies including butternuts were not cited. Most *C. moschata* studies were limited to single location experiments, which do not allow multi-location analysis.

## 2.8 Relationships between morpho-agronomic, internal quality and yield characteristics of butternut

### 2.8.1 Correlations

Correlations quantify the degree of genetic and non-genetic association between two or more traits. These associations are important for the early selection of plants or inbred lines and for the simultaneous selection of more than one desired trait at the same time (Silva *et al.*, 2016). Using phenotypic correlations could be risky since they could be influenced by environmental conditions. Genotypic correlations are usually stronger than phenotypic correlations, especially in cases where the environment influences the characteristics (Naik et al., 2015).

When traits express strong genetic correlations, it is possible to improve a certain trait by selecting the associated trait. This could be very beneficial when a trait has high economic value but low heritability. It would be easier to improve a high-value low-heritability trait by selecting an associated trait with a low-value but high-heritability. Similarly, in a case where traits with different degrees of difficulty for assessment are genetically correlated, the selection should be based on the traits easiest to evaluate (Grisales *et al.,* 2015). Therefore, information regarding genetic correlations among morpho-agronomic and quality characteristics, as well as yield and yield components, is required to improve selection efficiency (Nisha and Veeraragavathatham, 2014).

In crop improvement programmes yield is often the most important trait plant breeders will focus on. Similarly, studies of *C. moschata* have sought to establish associations between yield and various other characteristics. Studies involving tropical pumpkins confirmed that yield per plant was positively and significantly correlated with yield component traits, including average fruit weight, number of fruit per plant, fruit dimensions, flesh thickness, days to 50% flowering, vine length, plant biomass and leaf area. It was suggested that selection for these characteristics would be effective in improving yield (Aruah *et al.,* 2012; Tamilselvi *et al.,* 2012; Akter *et al.,* 2013; El-Tahawey *et al.,* 2015; Grisales *et al.,* 2015; Naik *et al.,* 2015; Mohsin *et al.,* 2017b). In both *C. moschata* and *C. maxima* yield has been negatively correlated to early flowering (Lawal, 2009; Mohsin *et al.,* 2017b). This implies that early flowering is detrimental to all yield parameters, since adequate time is required by the growing tropical pumpkin plant to generate photosynthesising organs for proper fruit development at a later stage.

After yield, quality is probably the second most important characteristic to improve in any plant breeding programme. Therefore, it is important to investigate the impact yield improvement may have on quality. Increased average fruit weight was negatively associated with DMC; therefore, selection for larger fruit will result in higher yield, but probably a lower DMC (Grisales *et al.,* 2015). The same study estimated yield to be positively associated with DMC and total carotene per plant, but negatively correlated with fruit starch. This association was beneficial since it is much cheaper to measure DMC than starch content. A negative association between carotene and DMC posed a challenge for genetic improvement since both play an important role in internal fruit quality. The positive correlation between yield and total carotenoid content was confirmed by Tamilselvi *et al.* (2012) and the positive correlation between yield and DMC by Lawal (2009). Yield has been both negatively (Akter *et al.,* 2013) and positively (Mohsin *et al.,* 2017b) correlated with TSS.

It was also established that flesh colour parameters measured in *L*a*b** and HSB (hue, saturation, brightness) had strong relationships with carotenoid profiles in both *C. moschata* and *C. maxima* (Seroczynska *et al.,* 2006; Itle and Kabelka, 2009). The *L** colour value had a negative correlation with lutein. A higher total carotenoid content, explained with a higher pigment concentration, would increase the darkness of the flesh colour, which is in agreement with a lower *L** value. The *a** colour value was positively associated with total carotenoid, lutein, β-carotene and α-carotene content. In sweet potato, β-carotene content was positively correlated with root flesh colour (Ameny and Wilson, 1997). Gurmu *et al.* (2018) obtained similar results and suggested that flesh colour in sweet potato could be potentially useful at the start of genotype screening where large numbers of progeny need to be evaluated and β-carotene analysis is not feasible.

No correlations between chlorophyll content and economically important traits in vegetable crops were cited. However, in both wheat (Araus *et al.,* 1998) and maize (Betran *et al.,* 2003), grain yield was positively correlated with chlorophyll fluorescence, suggesting greater radiation efficiency in genotypes with higher chlorophyll content.

### 2.8.2 Path coefficient analysis

Yield is a complex trait and is influenced by a number of component characteristics, which are interrelated among themselves. These interrelated associations influence their direct relationships with yield, and as a result, correlation coefficients are unreliable as selection indices. Often the indirect influences of one trait reduce the contribution of another trait with regard to yield. Unlike simple correlation analysis, path-coefficient analysis separates correlation coefficients into components of direct and indirect effects (Aruah *et al.,* 2012; Gurmu *et al.,* 2018). Path coefficient analysis is a standardised regression coefficient estimating the direct influence of a variable on another, regardless of other variables (Grisales *et al.,* 2015). This provides a comprehensive understanding of the associations among a set of characteristics and how each character affects or contributes to yield.

Results from a path coefficient analysis on *C. moschata* revealed that the maximum direct and positive contribution towards yield was obtained through fruit weight, fruit length, days to first female flower and number of fruit per vine (Naik *et al.,* 2015). Days to first male flower appearance and fruit diameter had negative direct effects on yield. In a separate study (Akter *et al.,* 2013), path coefficient analysis revealed that number of fruit per plant, days to first female flower and single fruit weight had maximum direct contributions towards yield and should be considered as primary yield components. Total sugars, number of female flowers per plant, reducing sugar and TSS content had a negative direct effect on yield.

Two other studies (Pandey *et al.,* 2008; Mohsin *et al.,* 2017b), which used path coefficient analysis indicated that fruit weight and number of fruit per plant showed maximum direct effects on yield at genotypic level, suggesting they were the main contributors. Direct selection based on these characteristics should result in significant improvement of yield. Lawal (2009) indicated with a path coefficient analysis of *C. maxima* that fruit mesocarp thickness was the largest contributor to yield.

Another study on *C. moschata* revealed that a higher plant dry matter per plant could be achieved by selection for a higher number of fruit per plant (Grisales *et al.,* 2015). However, fruit production per plant (fresh material) had a significant direct, but negative effect on plant dry matter per plant. This suggested that plant dry matter could be improved through selection for a high number of fruit or higher average fruit weight, rather than higher fruit production per plant. Heavy fruit with a low DMC resulted in a significant decrease in dry matter per plant. In addition, DMC had a positive direct effect on starch content but a negative indirect effect on total carotene content. Similar results were reported where DMC had an indirect relationship with carotenoid content but a direct relationship with starch content. Component breeding for starch and carotenoid content, respectively by reciprocal recurrent selection and backcrossing was suggested (Valdes-Restrepo *et al.,* 2013). Component breeding would be very effective where characteristics have a positive association. In the case of negative correlations, it would be difficult to improve both characteristics simultaneously through selection. In this case, it would be easier to improve these traits separately and recombine through hybridisation (El-Tahawey *et al.,* 2015).

Although fruit and roots are being utilised in butternut and sweet potato respectively, path coefficient analysis in both crops revealed that the number, average weight and the DMC of these storage organs had the highest positive direct effects on yield (Pandey *et al.,* 2008; Mohsin *et al.,* 2017b, Gurmu *et al.,* 2018). In okra, yield had a strong correlation with fruit weight and number of fruit, which was confirmed by path coefficient analysis (Reddy *et al.,* 2013).

Limited information is available on direct and indirect effects of plant morphological characteristics on yield in *C. moschata*. Results from agronomical crops might not be directly applicable to vegetable crops, but in cotton, path coefficient analysis showed that seed yield could be predicted by using leaf chlorophyll content under drought stress conditions (Karademir *et al.,* 2009).

In conclusion, crop improvement programmes depend mostly on the amount of genetic variability present in the population. Breeding success, however, is influenced by knowledge of key characteristics, their genetic mechanisms of inheritance, genetic and environmental factors influencing their expression and associations between characteristics. This information is critical when formulating an appropriate breeding strategy. Butternuts from Starke Ayres have gained market share in recent years but is still a relatively small market player compared to larger companies. In order to become more competitive, an effective approach to improve butternut internal quality, without sacrificing yield needs to be developed. Improvement of internal quality will not only benefit vegetable processors and household consumers but could also make a significant contribution to the nutritional requirements of small-scale farmers and communities in Africa.

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# CHAPTER 3

# PHENOTYPIC VARIABILITY OF MORPHO-AGRONOMIC AND INTERNAL FRUIT QUALITY CHARACTERISTICS OF BUTTERNUT


## 3.1 Abstract

The Starke Ayres *Cucurbita moschata* germplasm collection shows great diversity in morpho-agronomic and fruit quality characteristics, but these characteristics have not been comprehensively quantified to determine the true potential for the butternut squash breeding programme. The aim of this research was to quantify the phenotypic variability in morpho-agronomic and internal fruit quality characteristics of Starke Ayres butternut squash germplasm. The research was conducted using 42 selected genotypes including commercial hybrids, open-pollinated varieties, elite parental lines and test crosses between these parental lines. The screening trials were planted on three locations over two seasons where warm and dry environments were represented by Jacobsdal and Oudtshoorn and more humid and temperate conditions by Kaalfontein. Observations were recorded on 15 characteristics, which included leaf chlorophyll content, green-red and yellow-blue colour contribution in the leaf canopy, leaf width, petiole length, average fruit mass, dry matter yield, fruit number, fruit uniformity, yield, total soluble solids, dry matter content, green-red colour contribution in the fruit mesocarp, internal fruit breakdown and penetrometer readings as an indication of mesocarp firmness. The analysis of variance demonstrated highly significant differences among the genotypes for all characteristics. Location and/or season mean square differences were also significant for all traits, suggesting environmental influences. With the exception of chlorophyll content and leaf width, differential ranking of genotypes were noticed across environments for all characteristics supported by significant mean square differences for genotype x location x season interactions. In plant morphological traits, 14% to 33% of the phenotypic variability was attributed to genetic variation, in yield and yield component characteristics this value ranged from 16% to 62% and in fruit quality traits it ranged from 50% to 67%; suggesting the existence of vast inherent variability across the germplasm. Future improvement of butternut genotypes seems to be highly feasible through the exploitation of genetic variation in the current germplasm collection.



***Keywords*:** *Cucurbita moschata*, components of variance, genotypes, phenotypic diversity


## 3.2 Introduction

Butternut (*Cucurbita moschata)* belongs within the *Cucurbitaceae*, commonly referred to as the cucumber, gourd, melon and pumpkin family. *Cucurbita* is native to the Americas, with the

three most economically important species being *C. pepo, C. maxima* and *C. moschata. Cucurbita* species are warm-season annuals grown in almost all regions around the globe, from cool-temperate to tropical (Rolnik and Olas, 2020; Lopez-Anido, 2021).

*Cucurbita* are among the most morphologically variable genera in the plant kingdom. Phenotypic diversity in fruit shape, size, colour, productivity, internal fruit quality as well as various plant characteristics is high (Gomes *et al.*, 2020; Lee *et al.*, 2021). Although domestication of these species probably began 10 000 years ago, the current typical butternut fruit shape is a recent innovation (Mutschler and Pearson, 1987). Although vast species diversity has been described in literature (Du *et al.,* 2011; Aruah *et al.,* 2012; Akter *et al.,* 2013; Gamboa *et al.,* 2016), commercial butternut material available in South Africa has a narrow array of diversity and probably originated only from a few international breeding programmes.

*Cucurbita* plants are cultivated for various reasons, including animal and human consumption of mature and immature fruit, young leaves and shoots, as well as seed (Ferriol *et al.,* 2004). In South Africa, butternuts are grown mostly for human consumption of the mature fruit mesocarp. Data from the 19 largest fresh produce markets locally revealed butternut to be one of the most important vegetables in 2018. It ranked sixth after potato, tomato, onion, pepper and carrot for rand value (R288.9 million). Based on the mass sold on these markets, butternut was also in the sixth place, behind potato, onion, tomato, carrot and cabbage with 54 584 tons sold (DAFF, 2018). In less developed countries, *C. moschata* is also an important source of nutrients and occupies a prominent place amongst vegetables due to its high productivity, good storability, long period of availability and superior transport potential (Hazra *et al.,* 2007). However these characteristics can be influenced by the environment as well as morpho-agronomic characteristics.

Detailed descriptions of morpho-agronomic characteristics such as leaf colour and chlorophyll content, are not available in butternut literature. Although leaf colour in *C. moschata* has been described to vary from light to dark green (Kiramana and Isutsa, 2017) and is influenced by the presence or absence of silver mottling (Paris and Padley, 2014), the effect of leaf colour and other morpho-agonomic characteristics on butternut production is unknown. Although not cited in *C. moschata* literature, in barley (Kalaji and Guo, 2008) and cotton (Karademir *et al.,* 2009) high chlorophyll content has been linked to resistance to environmental stress, which has an indirect effect on yield and yield components. Genetic variation in various yield component characteristics has been described in *Cucurbita* (Pandey *et al*., 2008; Naik and Prasad, 2016) and was indicated to have an effect on internal fruit quality (Rana *et al.,* 2015; Restrepo-Salazar *et al.,* 2019). In *C. moschata*, fruit weight was reported to vary from 0.5 kg

(Gamboa *et al.*, 2016) to 10.3 kg (Abdein *et al*., 2017). Yield per plant is highly dependent on initial plant spacing, but a yield as high as 20.9 kg per plant has been reported (Restrepo *et al*., 2018).

Flesh colour, as an indication of internal quality, has been described to vary greatly in *Cucurbita*, but the effect on yield, and more specific butternut yield, is still unclear. Using the *L*a*b** (luminance and intensity) colour space, the value for fruit flesh have been measured ranging between 5.7 to 40.3 in *C. maxima* (Seroczynska *et al.*, 2006) and between -4.9 to 14.8 in *C. moschata* (Itle and Kabelka, 2009). Total soluble solids (TSS) has been recorded ranging from 4.7 to 17.2 °Brix (Carvalho *et al.*, 2015) and dry matter content (DMC) ranging from 6.1% (Zhang *et al.*, 2014) to 19.9% (Sojak *et al.,* 2014).

The aim of this study was to document the phenotypic variability of morpho-agronomic and internal fruit quality characteristics of Starke Ayres butternut squash germplasm. The variation available will be an indication of the scope for possible crop improvement through breeding.

### 3.3 Materials and methods

#### 3.3.1 Site description, trial design and management

Field experiments were conducted at three Starke Ayres research facilities within South Africa during the summers of 2018/2019 and 2019/2020. The Free State province facility is located on the farm Twee Riviere in the Jacobsdal area. Hartebeesfontein farm in the Kaalfontein area is located in the Gauteng province and Goedgeluk farm in Oudtshoorn is situated in the Western Cape province (Table 3.1). Both Jacobsdal and Oudtshoorn locations represent a very warm and dry environment with low disease pressure, while Oudtshoorn is much further south, resulting in longer day lengths. Kaalfontein is situated in a warm environment with frequent rain during the fruit maturation period and above average disease pressure. The climate data refer to mean daily maximum and minimum temperatures as well as hot days and cold nights that represent the average of the hottest day and coldest night of each month of the last 30 years. Only data from the butternut growing season months are presented.

Each of the locations hosted two plantings over two years, resulting in a total of six individual trials within growing seasons from October to March. All trials were laid out in a randomised complete block design with three replications at each location. In both 2018 and 2019 the trials were planted in October at all three locations by means of direct sowing of seed into raised beds (10 cm to 15 cm), covered with black plastic mulch and equipped with drip irrigation. Seeds were planted with an in-row spacing of 60 cm and between-row spacing of 180 cm. Plots were 720 cm in length to allow for 12 seeds per plot. After emergence, vines were

managed in such a way to cover the allocated area completely while still maintaining individual plot isolation. Trial management followed the same protocol for land preparation, fertilisation and weed management as what is recommended for commercial production of this crop (DAFF, 2011). Plant protection measures were taken when required.

**Table 3.1** Geographical description and long term wheather data for the summer butternut growing period of trial sites

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Kimberley** | | | | | | | |
| Latitude | 27°24'S | | | | | | |
| Longitude | 24°59'E | | | | | | |
| Altitude (m) | 1294 | | | | | | |
| | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
| Precipitation (mm) | 13 | 32 | 32 | 40 | 42 | 47 | 40 |
| Mean daily maximum (°C) | 26 | 29 | 31 | 33 | 33 | 32 | 30 |
| Hot days (°C) | 33 | 35 | 36 | 37 | 37 | 36 | 35 |
| Mean daily minimum (°C) | 7 | 11 | 14 | 17 | 18 | 18 | 15 |
| Cold nights (°C) | 0 | 3 | 6 | 10 | 12 | 11 | 8 |
| **Kaalfontein** | | | | | | | |
| Latitude | 26°01'S | | | | | | |
| Longitude | 28°19'E | | | | | | |
| Altitude (m) | 1578 | | | | | | |
| | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
| Precipitation (mm) | 17 | 71 | 104 | 124 | 127 | 88 | 75 |
| Mean daily maximum (°C) | 26 | 27 | 27 | 28 | 28 | 28 | 26 |
| Hot days (°C) | 31 | 33 | 33 | 33 | 33 | 33 | 31 |
| Mean daily minimum (°C) | 10 | 13 | 14 | 16 | 16 | 15 | 14 |
| Cold nights (°C) | 2 | 6 | 8 | 11 | 11 | 10 | 9 |
| **Oudtshoorn** | | | | | | | |
| Latitude | 33°31'S | | | | | | |
| Longitude | 22°02'E | | | | | | |
| Altitude (m) | 420 | | | | | | |
| | Sep | Oct | Nov | Dec | Jan | Feb | Mar |
| Precipitation (mm) | 14 | 32 | 27 | 21 | 15 | 23 | 28 |
| Mean daily maximum (°C) | 22 | 23 | 25 | 27 | 28 | 27 | 24 |
| Hot days (°C) | 32 | 34 | 35 | 36 | 37 | 36 | 34 |
| Mean daily minimum (°C) | 9 | 11 | 13 | 14 | 16 | 15 | 13 |
| Cold nights (°C) | 4 | 6 | 8 | 10 | 11 | 9 | 7 |

Sources: Google Earth (2021) and Meteoblue (2021)

### 3.3.2 Plant material

Since different geographical areas have different preferences, genotypes were selected from the Starke Ayres butternut germplasm bank so as to include maximum phenotypic variation. This included material developed in international breeding programmes of which some, but not all, are adapted to South African growing conditions. Some of these varieties suite South African market preferences, but based on external appearances, and in particular external colour, some do not meet requirements that are South African-specific. The material selected also included parental lines and hybrids from the Starke Ayres breeding programme developed under South African conditions, according to local market preferences. Seed of the Starke Ayres parental lines and specific $F_1$ crosses were produced on Kaalfontein in climate controlled plant production structures during the summer of 2016/2017. All plant material included in this study are listed in Table 3.2, with reference to the heritage, fruit shape, size and colour as well as country of origin. The first 24 genotypes were intended for line x tester analysis, and the remaining 18 were selected to incorporate a greater degree of variation with regard to plant morphology and internal fruit quality. Pluto F1 is used globally as a fresh market variety and has been coded in this study as entry G39 (HYB06).

### 3.3.3 Data collection

The first data on plant characteristics were collected 50 to 60 days after sowing during fruit set. An Opti-Sciences CCM-200 plus Chlorophyll Content Meter was used to determine chlorophyll content (CHL) in intact leaf samples. Ten random fully-developed leaves not showing any disease or physical damage were selected per plot. On each of these leaves, a single CHL reading was taken and used to calculate an average CHL reading per plot. A similar process was followed in selecting 10 random leaves with which to measure petiole length (PL) and leaf width (LW). The PL was taken as the distance from the vine attachment to the start of the leaf blade. The LW of the same leaf was measured at the widest point. Ten measurements of each of those characteristics were used to calculate plot averages.

**Table 3.2** List of butternut genotypes included in the trials for phenotypic evaluation

| Entry | Genotype | Heritage | Shape | Size | Colour | Country of origin |
|---|---|---|---|---|---|---|
| G1 | BUT01 | Tester line | Bell | Medium | Tan | South Africa |
| G2 | BUT02 | Tester line | Bell | Large | Tan | South Africa |
| G3 | BUT03 | Tester line | Bell | Medium | Tan | South Africa |
| G4 | BUT04 | Tester line | Bell | Large | Tan | South Africa |
| G5 | BUT05 | Elite parent line | Bell | Medium | Tan | South Africa |
| G6 | BUT06 | Elite parent line | Bell | Medium | Tan | South Africa |
| G7 | BUT07 | Elite parent line | Bell | Large | Tan | South Africa |
| G8 | BUT08 | Elite parent line | Bell | Small | Tan | South Africa |
| G9 | BUT01xBUT05 | Line tester cross | Bell | Large | Tan | South Africa |
| G10 | BUT02xBUT05 | Line tester cross | Bell | Large | Tan | South Africa |
| G11 | BUT03xBUT05 | Line tester cross | Bell | Large | Tan | South Africa |
| G12 | BUT04xBUT05 | Line tester cross | Bell | Large | Tan | South Africa |
| G13 | BUT01xBUT06 | Line tester cross | Bell | Small | Tan | South Africa |
| G14 | BUT02xBUT06 | Line tester cross | Bell | Small | Tan | South Africa |
| G15 | BUT03xBUT06 | Line tester cross | Bell | Small | Tan | South Africa |
| G16 | BUT04xBUT06 | Line tester cross | Bell | Medium | Tan | South Africa |
| G17 | BUT01xBUT07 | Line tester cross | Bell | Medium | Tan | South Africa |
| G18 | BUT02xBUT07 | Line tester cross | Bell | Medium | Tan | South Africa |
| G19 | BUT03xBUT07 | Line tester cross | Bell | Medium | Tan | South Africa |
| G20 | BUT04xBUT07 | Line tester cross | Bell | Medium | Tan | South Africa |
| G21 | BUT01xBUT08 | Line tester cross | Bell | Medium | Tan | South Africa |
| G22 | BUT02xBUT08 | Line tester cross | Bell | Medium | Tan | South Africa |
| G23 | BUT03xBUT08 | Line tester cross | Bell | Medium | Tan | South Africa |
| G24 | BUT04xBUT08 | Line tester cross | Bell | Medium | Tan | South Africa |
| G25 | BUT09 | F9 parent line | Goose neck | Extra large | Mottled green | Asia |
| G26 | BUT03xBUT09 | Specific $F_1$ cross | Goose neck | Large | Mottled green | Asia x South Africa |
| G27 | BUT06xBUT09 | Specific $F_1$ cross | Goose neck | Extra large | Mottled green | Asia x South Africa |
| G28 | BUT10xBUT11 | Specific $F_1$ cross | Goose neck | Large | Mottled green | Asia x South Africa |
| G29 | BUT12 | F9 parent line | Bell | Large | Tan | South Africa |
| G30 | HYB01 | Commercial $F_1$ | Bell | Large | Tan | South Africa |
| G31 | BUT13 | F9 parent line | Bell | Small | Tan | USA |
| G32 | BUT14 | F9 parent line | Bell | Large | Tan | South Africa |
| G33 | BUT15 | F9 parent line | Bell | Medium | Tan | South Africa |
| G34 | OPV01 | OP variety | Bell | Medium | Orange-brown | Spain |
| G35 | HYB02 | Commercial $F_1$ | Bell | Extra large | Tan | South Africa |
| G36 | HYB03 | Commercial $F_1$ | Bell | Medium | Tan | China |
| G37 | HYB04 | Commercial $F_1$ | Bell | Medium | Tan | Brazil |
| G38 | HYB05 | Commercial $F_1$ | Bell | Medium | Tan | Brazil |
| G39 | HYB06 | Commercial $F_1$ | Bell | Medium | Tan | Brazil |
| G40 | OPV02 | OP variety | Bell | Medium | Orange-brown | Argentina |
| G41 | HYB07 | Commercial $F_1$ | Bell | Extra large | Tan | Brazil |
| G42 | HYB08 | Commercial $F_1$ | Bell | Small | Tan | South Africa |

Leaf canopy colour quantification was accomplished by means of digital image analysis. Since data-capturing was conducted under field conditions, standardisation of image-illumination was challenging. Images were captured between 11:00 and 13:00 in order to minimise potential shading on clear-sky days and to prevent variation in light intensity due to cloud movement. The camera was positioned 100 cm from the leaf canopy and three random images of each plot were taken with a Sony Cybershot DSC-WX300 colour digital camera. Auto mode was used but aperture (f = 3.5) and exposure (1/250 seconds) were consistent throughout the period of generating these images. Resolution of 3648 x 2736 pixels was used and images stored in JPEG format. Digital analysis followed using ImageJ software to obtain red, green and blue (RGB) colour space values for quantification of the average leaf canopy colour for each image (Marakami *et al.*, 2005). Since RGB values are device-dependant, it should be mentioned that all images in this study were taken with the same equipment with standardised settings.

All RGB values were converted to the more universally acceptable *L*a*b** colour space, which matches human perception of colour (Mendoza *et al.,* 2006). Values were also converted to hue, saturation and brightness referred to as the HSB colour model. Both conversions were done using Colour Conversion Centre 4.0c (Boronkay, 2010). The a* (green-red spectrum) and b* (yellow-blue spectrum) values represented the data better based on visual appearance and were the only values used in the analysis.

At 60 days past flowering, mature fruit were harvested which resulted in the yield component characteristics. All fruit from a specific plot were weighed (which will be referred to as yield) and the number of fruit per plot (FN) counted. These values were used to calculate average fruit mass (AFM) per plot. Although specific genotypes have typical fruit shapes, butternut fruit shape is known to be influenced by the environment. The number of fruit in a plot corresponding with the typical fruit shape, expressed as a percentage, was used as an indication of uniformity. Greater percentages represented greater uniformity.

Five fruit were randomly selected from each plot and stored for two months under ambient conditions and allowed to fully mature, with total carotenoid and DMC levels rising before internal quality assessments were completed. All five fruit were included in the analysis for a representative average per plot.

Mesocarp slices were obtained from the elongated butternut fruit necks within the equatorial fruit zone at least 2 cm proximal to the seed cavity (Figure 3.1). These fruit slices were cut and

peeled using stainless steel knives and measurements were completed immediately so as to prevent moisture loss from the surface, which could potentially influence colour and quality.

Penetrometer readings (PEN) were taken using a Fruit Pressure Tester model FT 327 with a 3.0 mm shaft diameter penetrated to a depth of 15 mm and was used as an indication of mesocarp (flesh) firmness. After a cross section of the fruit, two penetrometer readings were taken, on opposite sides of the fruit, one third removed from the outside (Figure 3.1c).

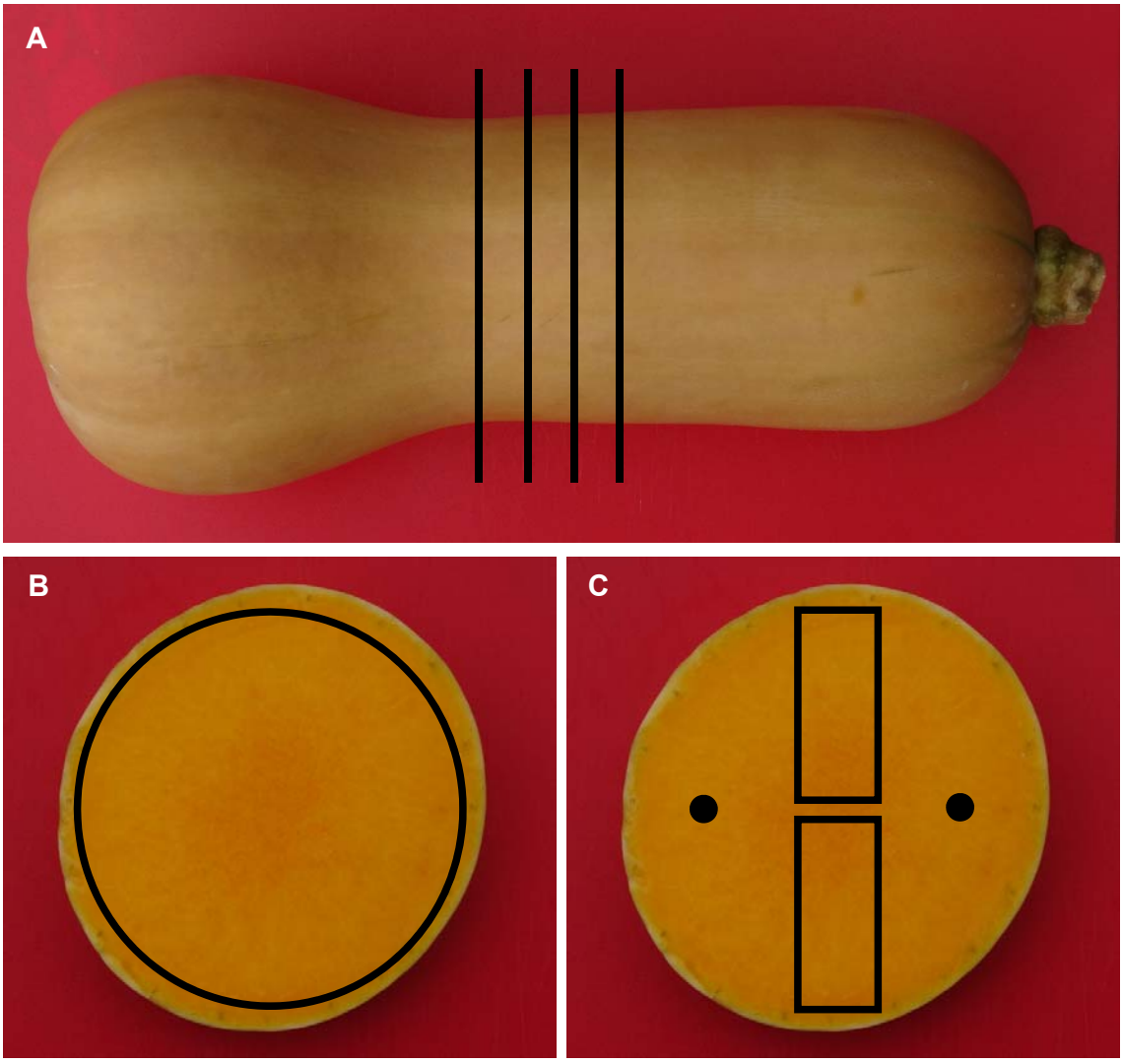


**Figure 3.1** Different sampling areas of butternut fruit for internal quality assessment after two months of storage. A: Slices used for internal fruit quality assessments, B: Flesh colour analysis (inside of the circle), C: Penetrometer readings (circles); Total soluble solids (rectangular)

Digital images were taken in order to quantify flesh colour. The Sony DSC-WX300 camera was mounted on the outside of a carton box with the lens protruding through the top surface. This allowed stable support with easy access to the camera controls. All sides of the box were closed to exclude varying light intensities in the environment, with the exception of one side as a working entrance. The samples were illuminated using an external light source (Lumaglo A60 ES LED Globe, AC 230V 50 Hz 9W, Non-dimmable Cool white) mounted on the inside of the box at an angle to give uniform standard illumination on all samples. Since the flesh samples had a flat cut surface, samples were homogeneous with limited colour variation within a sample. The camera was positioned 50 cm vertically above the object using a red plastic sheet as background. Only one image was taken per fruit, and it covered the entire cross section of the fruit neck mesocarp (Figure 3.1b). Images were captured using an auto function with the zoom at 3.0 with the flash function off. The images were captured with a lens aperture at *f* = 4.6, speed at 1/80 seconds, resolution of 3648 x 2736 pixels and images were stored in JPEG format using sRGB colour representation. The images were transferred to a laptop computer and analysed as described above for canopy colour. Based on visual interpretation higher a* values represented darker red-orange colour and was the only values used in the analysis.

From each fruit a 1.5 cm wide section was cut through the centre from a 1.5 cm to 2.0 cm thick fruit slice (Figure 3.1.c). This section was cut in half, resulting in two samples from opposite sides of the neck, each with a volume of 6.5 $cm^2$ to 9.0 $cm^2$. The samples were frozen for 48 hours and then thawed at room temperature, permitting a reading of TSS after sap was squeezed onto a digital Atago PAL-1 pocket refractometer (Adeeko *et al.*, 2020). TSS readings were taken in duplicate for each fruit.

An additional sample of fruit flesh of approximately 130 g was sliced into 0.5 cm thick slices. The initial weight was recorded and dried until constant weight remained, which was recorded as the dry mass. The recommended maximum temperature for drying of vegetables for human consumption is 60°C in a tunnel drier (Sojak *et al.,* 2014; Dhiman *et al.,* 2017). In this experiment the material was placed on multiple trays in single layers and dried under forced air at 55°C. It took approximately 48 hours to reach a constant weight. All weight measurements were taken using a digital balance with an accuracy of 0.01 g. The DMC was expressed as a percentage of the fresh weight using the formula:

$$\mathrm{DMC} = M_f / M_i \times 100$$

Where $M_f$ is the flesh mass after drying and $M_i$ is the initial flesh mass before drying (Zaccari and Galietta, 2015). The product of DMC and yield was referred to as dry matter yield (DMY) as an estimate of the total dried fruit mesocarp produced per plot. In this study, the seeds and fruit rinds were not taken into consideration.

### 3.3.4 Statistical analysis

To estimate the variability of the various traits among butternut genotypes, an analysis of variance (ANOVA) was performed for each location, and genotypic means were compared by the least significant difference (LSD) test using P≤0.05. The effect of the three environments (Jacobsdal, Kaalfontein and Oudtshoorn) and the genotype x environment interaction were analysed with combined ANOVA across seasons and locations, considering genotypes as fixed effects. All statistical analyses were performed using Genstat® for Windows, 19$^{th}$ edition (VSN International, 2017).

## 3.4 Results

The combined ANOVA across three locations and two successive seasons showed highly significant (P≤0.001) differences among the 42 genotypes for all 15 morpho-agronomic traits evaluated (Table 3.3). The mean square values for plant characteristics [CHL, green-red colour contribution in the leaves (Leaf a*), yellow-blue colour contribution in leaves (Leaf b*), LW and PL] are presented in Table 3.3. The mean square values for the primary sources of variation viz. genotype (entry), location (loc) and season, were all highly significant, but with most of the variation attributed to genotypes and locations where the percentage of the variation in both instances was mostly higher than 20%. In the case of leaf colour (Leaf a* and Leaf b*) more than 50% of the total variation was attributed to differences in location. Sources of variation due to interactions: entry x location and location x season, were also highly significant, but contributed very little to the total amount of variation. It was only for LW (21.57%) and PL (27.03%) where location x season contributed substantially to total variation.

**Table 3.3** Combined analysis of variance and contribution of main effects to the variation within leaf chlorophyll content (CHL), green-red colour contribution in leaves (Leaf a*), yellow-blue colour contribution in leaves (Leaf b*), leaf width (LW) and petiole length (PL) across three environments in two growing seasons

| | | CHL | | | Leaf a* | | | Leaf b* | | | LW | | | PL | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | MS | | % Var | MS | | % Var | MS | | % Var | MS | | % Var | MS | | % Var |
| Replication | 2 | 163.97 | | 0.77 | 9.97 | | 0.14 | 11.77 | | 0.13 | 4719.20 | | 1.18 | 5578.50 | | 0.50 |
| Entry | 41 | 259.59 | *** | 25.02 | 49.63 | *** | 14.36 | 107.70 | *** | 23.99 | 5523.50 | *** | 28.30 | 17847.10 | *** | 32.68 |
| Loc | 2 | 8159.37 | *** | 38.37 | 3550.53 | *** | 50.12 | 5395.77 | *** | 58.64 | 93874.40 | *** | 23.46 | 196224.90 | *** | 17.53 |
| Season | 1 | 2108.71 | *** | 4.96 | 1645.57 | *** | 11.62 | 248.81 | *** | 1.35 | 13626.30 | *** | 1.70 | 5150.00 | ** | 0.23 |
| Entry.Loc | 82 | 28.30 | *** | 5.46 | 8.10 | *** | 4.69 | 6.12 | *** | 2.73 | 474.30 | *** | 4.86 | 1385.90 | *** | 5.08 |
| Entry.Season | 41 | 21.14 | * | 2.04 | 4.57 | * | 1.32 | 3.82 | NS | 0.85 | 283.00 | NS | 1.45 | 872.10 | * | 1.60 |
| Loc.Season | 2 | 709.19 | *** | 3.33 | 294.33 | *** | 4.16 | 85.72 | *** | 0.93 | 86322.80 | *** | 21.57 | 302642.70 | *** | 27.03 |
| Entry.Loc.Season | 82 | 18.18 | NS | 3.51 | 4.68 | ** | 2.71 | 4.74 | * | 2.11 | 216.80 | NS | 2.22 | 767.20 | * | 2.81 |
| Residual | 502 | 14.02 | | | 3.07 | | | 3.40 | | | 243.10 | | | 559.80 | | |
| Total | 755 | | | | | | | | | | | | | | | |
| CV% | | 9.00 | | | 9.10 | | | 7.00 | | | 5.40 | | | 6.90 | | |
| $R^2$ | | 0.83 | | | 0.89 | | | 0.91 | | | 0.85 | | | 0.87 | | |

*P≤0.05, **P≤0.01, ***P≤0.001, NS: Not significant, df: Degrees of freedom, MS: Mean squares, CV: Coefficient of variation, Loc: Location, % Var: Percentage of total sum of squares, $R^2$: Coefficient of determination

The mean square values for AFM, DMY, FN, fruit uniformity and yield (Table 3.4) revealed highly significant (P≤0.001) differences for all primary and all interaction sources of variance, except season for uniformity which was significant (P≤0.01). Genotypes were the main contributor to the variance for AFM (62.5%), FN (52.8%) and uniformity (38.8%). Location x season interactions (31.4%) and genotype (28.3%) made similar contributions to the total variation within yield. Location x season interactions contributed 39.9% of the total DMY variation, compared to contributions of 16.2% and 18.0% from the genotypes and locations, respectively. Location contributed 20.4% of the total AFM variation.

All mean square values derived from aspects of internal quality [TSS, DMC, green-red colour contribution in the fruit mesocarp (Fruit a*), internal breakdown (IBD) and PEN] were significant, with the exception of seasonal effects on TSS and location effect on Fruit a* (Table 3.5). The influence of genotype on variance was by far the greatest, contributing more than half for all characteristics.

The top 10 and lowest 10 genotypes ranked for each of the morpho-agronomic and internal fruit quality characteristics are highlighted in green and orange, respectively (Tables 3.6 to 3.8). Considerable variability exists among the genotypes as indicated by large differences between minimum and maximum values in this study. South African commercial varieties (hereafter referred to as commercial varieties) were all spread over the entire CHL, Leaf a*, Leaf b* and PL ranges (Table 3.6). LW for the commercial hybrids were not significantly different from the mean, except for G30, which was significantly lower.

Leaf a* and Leaf b* ranged respectively from -22.6 to -15.6 and 20.8 to 30.0. Fifteen of the genotypes had opposite rankings for Leaf a* and Leaf b*, in which a high ranking for Leaf a* was paired with a low ranking for Leaf b* and *vice versa*. LW ranged from 241.8 mm to 327.3 mm and PL from 263.4 mm to 431.4 mm. Genotype G35, a leading variety used for processing, ranked low for CHL and Leaf a* but very high for Leaf b* and PL. The opposite was true for G38 (a leading variety used for shipping), which ranked high for CHL and Leaf a* but low for Leaf b* and PL. Genotype G42 ranked high for CHL and PL but low for Leaf a*. These results indicated an almost random performance of the commercial varieties with regard to different plant characteristics, with no clear patterns emerging. All butternut types and market segments were distributed across the great diversity of all the plant characteristics evaluated.

**Table 3.4** Combined analysis of variance and contribution of main effects to the variation within average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), fruit uniformity and yield across three environments in two growing seasons

| | | AFM | | DMY | | FN | | Uniformity | | Yield | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | MS | % Var | MS | % Var | MS | % Var | MS | % Var | MS | % Var |
| Replication | 2 | 0.08 | 0.03 | 29.65 | 0.67 | 391.96 | 0.25 | 79.89 | 0.15 | 772.10 | 0.20 |
| Entry | 41 | 7.07 *** | 62.46 | 34.94 *** | 16.15 | 4116.49 *** | 52.79 | 1027.16 *** | 38.78 | 5368.80 *** | 28.29 |
| Loc | 2 | 47.26 *** | 20.38 | 798.45 *** | 18.00 | 8329.96 *** | 5.21 | 1121.56 *** | 2.07 | 56231.70 *** | 14.46 |
| Season | 1 | 1.81 *** | 0.39 | 502.63 *** | 5.67 | 4665.19 *** | 1.46 | 593.78 ** | 0.55 | 42333.50 *** | 5.44 |
| Entry.Loc | 82 | 0.30 *** | 5.27 | 3.58 *** | 3.31 | 170.64 *** | 4.38 | 142.29 *** | 10.74 | 270.70 *** | 2.85 |
| Entry.Season | 41 | 0.25 *** | 2.21 | 4.09 *** | 1.89 | 167.41 *** | 2.15 | 171.56 *** | 6.48 | 481.10 *** | 2.54 |
| Loc.Season | 2 | 6.13 *** | 2.64 | 1771.74 *** | 39.94 | 31634.79 *** | 19.79 | 2004.10 *** | 3.69 | 121955.40 *** | 31.35 |
| Entry.Loc.Season | 82 | 0.13 *** | 2.26 | 3.69 *** | 3.41 | 163.46 *** | 4.19 | 123.75 *** | 9.34 | 426.40 *** | 4.49 |
| Residual | 502 | 0.04 | | 1.94 | | 62.29 | | 61.04 | | 160.80 | |
| Total | 755 | | | | | | | | | | |
| CV% | | 11.80 | | 19.10 | | 17.00 | | 14.00 | | 17.90 | |
| $R^2$ | | 0.96 | | 0.89 | | 0.90 | | 0.72 | | 0.90 | |

**P≤0.01, ***P≤0.001, df: Degrees of freedom, MS: Mean squares, CV: Coefficient of variation, Loc: Location, % Var: Percentage of total sum of squares, $R^2$: Coefficient of determination

**Table 3.5** Combined analysis of variance and contribution of main effects to the variation within total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) across three environments in two growing seasons

| | | TSS | | DMC | | Fruit a* | | IBD | | PEN | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | MS | % Var | MS | % Var | MS | % Var | MS | % Var | MS | % Var |
| Replication | 2 | 0.28 | 0.03 | 15.39 | 0.85 | 28.10 | 0.28 | 694.34 | 0.69 | 0.46 | 0.54 |
| Entry | 41 | 28.59 *** | 56.16 | 51.79 *** | 58.54 | 327.23 *** | 67.31 | 2474.55 *** | 50.70 | 2.31 *** | 54.97 |
| Loc | 2 | 55.33 *** | 5.30 | 34.76 *** | 1.92 | 15.03 NS | 0.15 | 4971.32 *** | 4.97 | 2.94 *** | 3.41 |
| Season | 1 | 0.35 NS | 0.02 | 7.53 ** | 0.21 | 85.80 *** | 0.43 | 1466.96 *** | 0.73 | 18.30 *** | 10.61 |
| Entry.Loc | 82 | 1.58 *** | 6.20 | 2.39 *** | 5.41 | 12.53 *** | 5.16 | 229.61 *** | 9.41 | 0.10 *** | 4.85 |
| Entry.Season | 41 | 1.43 *** | 2.80 | 2.40 *** | 2.71 | 17.16 *** | 3.53 | 183.42 *** | 3.76 | 0.09 ** | 2.03 |
| Loc.Season | 2 | 75.85 *** | 7.27 | 194.73 *** | 10.74 | 536.55 *** | 5.38 | 2374.95 *** | 2.37 | 4.14 *** | 4.79 |
| Entry.Loc.Season | 82 | 1.52 *** | 5.96 | 1.88 *** | 4.25 | 8.54 ** | 3.51 | 16.497 *** | 6.86 | 0.08 ** | 3.78 |
| Residual | 502 | 0.68 | | 1.11 | | 5.66 | | 81.74 | | 0.05 | |
| Total | 755 | | | | | | | | | | |
| CV% | | 10.30 | | 10.20 | | 13.30 | | 11.6 | | 8.60 | |
| $R^2$ | | 0.84 | | 0.85 | | 0.86 | | 0.79 | | 0.85 | |

**P≤0.01, ***P≤0.001, NS: Not significant, df: Degrees of freedom, MS: Mean squares, CV: Coefficient of variation, Loc: Location, % Var: Percentage of total sum of squares, $R^2$: Coefficient of determination

In contrast to plant characteristics (Table 3.6), yield component characteristics are largely linked to market segments. AFM ranged from 0.8 kg to 4.0 kg, with processing genotypes (AFM larger than 2.0 kg) ranking very high and shipping varieties (AFM smaller than 1.3 kg) ranking very low (Table 3.7). The genotype with the highest AFM was G25 (4.0 kg), which is almost double that of commercial processing varieties G35 and G41.

The combined ANOVA, AFM showed limited variation attributed to interactions, suggesting rankings to be consistent across seasons and locations even though genotypes, locations and seasons were all significantly different. Most of the larger-fruited genotypes ranked high for yield but low for FN. The highest yield across locations was recorded for a large-fruited genotype, G9 at 96.0 kg. This hybrid was not significantly different from the commercial processor G35, or hybrids G23, G21 and G22. The latter three hybrids fitted surprisingly into a different market segment due to significantly smaller fruit sizes.

FN ranged from 9.6 to 76.0, with G42 yielding most fruit per plot and it was significantly different from all other genotypes (Table 3.7). It was also the only commercially available genotype ranked in the top 10 for DMY and not significantly lower than the top performer G15. Uniformity ranged from 41.1% to 67.8%. Four of the commercial varieties (G35, G39, G37 and G38) were ranked in the top 10 for uniformity with the remaining three (G41, G42 and G30) ranking in the bottom 10.

Even though exceptional internal fruit quality is not important for the South African butternut industry, substantial variation was found for TSS, DMC, Fruit a*, IBD and PEN values (Table 3.8). Currently the only characteristic known to the domestic consumer is flesh colour, but since the grower determines variety selection, this is of minor importance. Butternut flesh colour ranges from yellow-orange to red-orange, with the latter being preferred. In this study Fruit a* as an indication of the amount of red in a sample ranged from 5.7 to 27.6. G31 was the highest ranking genotype and it was significantly different from all commercial material. G42 was the best performing commercial hybrid for Fruit a*. The worst performer was G25, which is known to have a pale to almost white flesh. The hybrids related to this parent also ranked very low. G5 ranked very low for flesh colour and this poor performance could possibly be attributed to its later maturing tendency.

**Table 3.6** Mean values and rankings of leaf chlorophyll content (CHL), green-red colour contribution in leaves (Leaf a*), yellow-blue colour contribution in leaves (Leaf b*), leaf width (LW) and petiole length (PL) evaluated across three environments and two growing seasons

| | | CHL | | Leaf a* | | Leaf b* | | LW (mm) | | PL (mm) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Entry | Genotype | Mean | Rank | Mean | Rank | Mean | Rank | Mean | Rank | Mean | Rank |
| G1 | BUT01 | 42.47 | 20 | -18.96 | 29 | 28.02 | 10 | 257.39 | 40 | 340.64 | 22 |
| G2 | BUT02 | 37.69 | 34 | -20.57 | 32 | 29.04 | 4 | 254.88 | 41 | 332.41 | 26 |
| G3 | BUT03 | 40.20 | 27 | -20.76 | 35 | 27.72 | 12 | 283.36 | 33 | 309.72 | 35 |
| G4 | BUT04 | 40.00 | 29 | -15.55 | 1 | 20.76 | 42 | 303.33 | 8 | 263.44 | 42 |
| G5 | BUT05 | 30.24 | 42 | -17.88 | 10 | 23.91 | 33 | 327.25 | 1 | 296.08 | 40 |
| G6 | BUT06 | 43.97 | 13 | -19.55 | 20 | 25.82 | 29 | 292.91 | 18 | 299.91 | 39 |
| G7 | BUT07 | 37.33 | 36 | -21.45 | 40 | 28.27 | 7 | 291.06 | 23 | 338.11 | 24 |
| G8 | BUT08 | 41.98 | 23 | -21.07 | 39 | 29.99 | 1 | 241.75 | 42 | 302.50 | 38 |
| G9 | BUT01xBUT05 | 39.38 | 31 | -19.12 | 15 | 26.67 | 21 | 285.06 | 30 | 339.78 | 23 |
| G10 | BUT02xBUT05 | 37.41 | 35 | -20.50 | 31 | 27.64 | 13 | 291.67 | 21 | 355.06 | 14 |
| G11 | BUT03xBUT05 | 41.43 | 24 | -19.77 | 23 | 26.10 | 25 | 310.10 | 5 | 324.05 | 29 |
| G12 | BUT04xBUT05 | 36.65 | 40 | -17.85 | 9 | 23.33 | 35 | 319.58 | 2 | 294.50 | 41 |
| G13 | BUT01xBUT06 | 46.37 | 4 | -18.37 | 12 | 26.18 | 23 | 284.11 | 32 | 357.67 | 12 |
| G14 | BUT02xBUT06 | 44.83 | 9 | -18.33 | 11 | 25.86 | 28 | 285.00 | 31 | 349.11 | 18 |
| G15 | BUT03xBUT06 | 48.20 | 2 | -18.55 | 13 | 24.87 | 32 | 297.33 | 14 | 334.89 | 25 |
| G16 | BUT04xBUT06 | 45.78 | 6 | -17.73 | 8 | 23.63 | 34 | 302.72 | 9 | 308.89 | 36 |
| G17 | BUT01xBUT07 | 42.65 | 18 | -20.27 | 29 | 27.58 | 15 | 287.44 | 29 | 363.72 | 10 |
| G18 | BUT02xBUT07 | 38.86 | 32 | -20.16 | 26 | 27.35 | 17 | 290.56 | 25 | 370.94 | 8 |
| G19 | BUT03xBUT07 | 41.07 | 25 | -20.26 | 28 | 26.62 | 22 | 300.86 | 11 | 350.72 | 17 |
| G20 | BUT04xBUT07 | 40.93 | 26 | -19.66 | 22 | 25.62 | 30 | 315.61 | 3 | 324.00 | 30 |
| G21 | BUT01xBUT08 | 42.13 | 21 | -20.59 | 33 | 28.99 | 5 | 261.89 | 39 | 351.06 | 16 |
| G22 | BUT02xBUT08 | 43.00 | 14 | -20.23 | 27 | 28.40 | 6 | 268.17 | 38 | 365.83 | 9 |
| G23 | BUT03xBUT08 | 42.99 | 16 | -20.36 | 30 | 27.48 | 16 | 269.92 | 37 | 343.44 | 21 |
| G24 | BUT04xBUT08 | 44.03 | 12 | -19.40 | 17 | 26.00 | 27 | 289.27 | 27 | 329.36 | 27 |
| G25 | BUT09 | 36.67 | 39 | -20.96 | 38 | 28.12 | 8 | 274.83 | 34 | 382.28 | 4 |
| G26 | BUT03xBUT09 | 38.80 | 33 | -19.29 | 16 | 27.88 | 11 | 270.94 | 35 | 383.61 | 2 |
| G27 | BUT06xBUT09 | 41.99 | 22 | -20.90 | 37 | 26.78 | 20 | 312.17 | 4 | 431.44 | 1 |
| G28 | BUT10xBUT11 | 37.25 | 37 | -19.51 | 19 | 25.51 | 31 | 302.50 | 10 | 382.67 | 3 |
| G29 | BUT12 | 39.70 | 30 | -19.47 | 18 | 26.88 | 19 | 306.50 | 6 | 360.11 | 11 |
| G30 | HYB01# | 44.10 | 11 | -19.65 | 21 | 28.06 | 9 | 270.26 | 36 | 351.70 | 15 |
| G31 | BUT13 | 45.08 | 8 | -22.64 | 42 | 29.87 | 2 | 296.89 | 15 | 344.00 | 20 |
| G32 | BUT14 | 36.86 | 38 | -20.75 | 34 | 27.60 | 14 | 298.00 | 12 | 324.33 | 28 |
| G33 | BUT15 | 42.58 | 19 | -20.06 | 25 | 26.18 | 23 | 303.39 | 7 | 355.72 | 13 |
| G34 | OPV01 | 44.77 | 10 | -16.39 | 4 | 21.34 | 41 | 292.28 | 20 | 315.89 | 33 |
| G35 | HYB02# | 35.17 | 41 | -21.68 | 41 | 29.68 | 3 | 291.44 | 22 | 371.00 | 7 |
| G36 | HYB03 | 45.25 | 7 | -16.26 | 2 | 22.09 | 39 | 290.06 | 26 | 316.50 | 32 |
| G37 | HYB04# | 42.82 | 17 | -16.81 | 6 | 22.37 | 37 | 293.33 | 17 | 378.94 | 5 |
| G38 | HYB05# | 45.86 | 5 | -16.76 | 5 | 22.33 | 38 | 287.85 | 28 | 312.22 | 34 |
| G39 | HYB06# | 43.00 | 14 | -17.51 | 7 | 22.94 | 36 | 292.60 | 19 | 307.24 | 37 |
| G40 | OPV02 | 46.86 | 3 | -16.28 | 3 | 21.70 | 40 | 297.39 | 13 | 324.00 | 30 |
| G41 | HYB07# | 40.16 | 28 | -19.95 | 24 | 26.02 | 26 | 291.05 | 24 | 347.44 | 19 |
| G42 | HYB08# | 48.27 | 1 | -20.77 | 36 | 27.25 | 18 | 295.06 | 16 | 378.22 | 6 |
| | Mean | 41.54 | | -19.35 | | 26.15 | | 289.95 | | 340.79 | |
| | Minimum | 30.24 | | -22.64 | | 20.76 | | 241.75 | | 263.44 | |
| | Maximum | 48.27 | | -15.55 | | 29.99 | | 327.25 | | 431.44 | |
| | LSD | 2.45 | | 1.15 | | 1.21 | | 10.21 | | 15.50 | |
| | CV% | 9.00 | | 9.10 | | 7.00 | | 5.40 | | 6.90 | |

# Commercially available in South Africa; LSD: Least significant difference, CV: Coefficient of variation; Top 10 and bottom 10 ranked genotypes are highlited in green and orange respectively

**Table 3.7** Mean values and rankings of average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), fruit uniformity and yield evaluated across three environments and two growing seasons

| | | AFM (kg) | | DMY (%) | | FN | | Uniformity (%) | | Yield (kg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Entry | Genotype | Mean | Rank | Mean | Rank | Mean | Rank | Mean | Rank | Mean | Rank |
| G1 | BUT01 | 1.07 | 38 | 7.40 | 24 | 64.17 | 5 | 47.78 | 35 | 69.34 | 24 |
| G2 | BUT02 | 1.26 | 31 | 6.66 | 30 | 51.67 | 17 | 41.11 | 42 | 65.03 | 28 |
| G3 | BUT03 | 1.23 | 34 | 7.45 | 23 | 51.22 | 18 | 62.78 | 10 | 62.14 | 31 |
| G4 | BUT04 | 1.48 | 22 | 5.49 | 39 | 41.22 | 24 | 65.56 | 5 | 60.06 | 32 |
| G5 | BUT05 | 2.42 | 5 | 2.30 | 42 | 9.61 | 42 | 51.67 | 27 | 25.80 | 42 |
| G6 | BUT06 | 1.06 | 39 | 5.97 | 35 | 35.83 | 34 | 50.56 | 32 | 37.49 | 41 |
| G7 | BUT07 | 1.81 | 15 | 6.22 | 33 | 22.33 | 40 | 55.00 | 22 | 38.81 | 40 |
| G8 | BUT08 | 0.97 | 41 | 5.15 | 40 | 54.11 | 14 | 65.00 | 6 | 51.01 | 36 |
| G9 | BUT01xBUT05 | 2.39 | 6 | 8.94 | 3 | 40.94 | 26 | 56.11 | 19 | 95.97 | 1 |
| G10 | BUT02xBUT05 | 2.67 | 3 | 8.42 | 8 | 36.33 | 33 | 51.11 | 28 | 93.97 | 2 |
| G11 | BUT03xBUT05 | 2.30 | 7 | 8.34 | 12 | 39.56 | 28 | 51.11 | 28 | 86.99 | 10 |
| G12 | BUT04xBUT05 | 2.59 | 4 | 6.22 | 33 | 26.33 | 39 | 54.44 | 23 | 65.44 | 26 |
| G13 | BUT01xBUT06 | 1.13 | 37 | 8.79 | 5 | 68.67 | 2 | 53.89 | 24 | 78.24 | 17 |
| G14 | BUT02xBUT06 | 1.24 | 33 | 8.34 | 12 | 63.83 | 6 | 51.11 | 28 | 78.81 | 16 |
| G15 | BUT03xBUT06 | 1.23 | 34 | 9.15 | 1 | 63.67 | 8 | 61.67 | 12 | 76.90 | 18 |
| G16 | BUT04xBUT06 | 1.42 | 27 | 8.55 | 6 | 56.33 | 12 | 61.11 | 13 | 79.79 | 15 |
| G17 | BUT01xBUT07 | 1.66 | 17 | 8.81 | 4 | 45.78 | 21 | 49.44 | 34 | 73.49 | 21 |
| G18 | BUT02xBUT07 | 1.89 | 13 | 8.39 | 10 | 40.11 | 27 | 52.78 | 25 | 74.51 | 20 |
| G19 | BUT03xBUT07 | 1.71 | 16 | 7.79 | 20 | 39.00 | 31 | 62.78 | 10 | 65.10 | 27 |
| G20 | BUT04xBUT07 | 1.91 | 12 | 7.55 | 21 | 35.06 | 35 | 60.00 | 15 | 64.76 | 29 |
| G21 | BUT01xBUT08 | 1.35 | 28 | 8.37 | 11 | 68.06 | 3 | 57.78 | 18 | 89.86 | 7 |
| G22 | BUT02xBUT08 | 1.49 | 20 | 7.87 | 18 | 62.44 | 9 | 58.33 | 17 | 91.30 | 4 |
| G23 | BUT03xBUT08 | 1.48 | 22 | 8.47 | 7 | 63.78 | 7 | 66.67 | 4 | 90.73 | 5 |
| G24 | BUT04xBUT08 | 1.59 | 19 | 7.99 | 16 | 52.50 | 15 | 67.22 | 3 | 82.84 | 13 |
| G25 | BUT09 | 4.00 | 1 | 5.52 | 38 | 13.94 | 41 | 60.56 | 14 | 52.75 | 35 |
| G26 | BUT03xBUT09 | 2.07 | 10 | 8.10 | 15 | 45.28 | 22 | 58.89 | 16 | 92.56 | 3 |
| G27 | BUT06xBUT09 | 3.03 | 2 | 9.13 | 2 | 29.83 | 37 | 55.56 | 20 | 89.31 | 8 |
| G28 | BUT10xBUT11 | 1.92 | 11 | 8.18 | 14 | 46.22 | 20 | 46.67 | 37 | 88.04 | 9 |
| G29 | BUT12 | 1.63 | 18 | 5.01 | 41 | 29.00 | 38 | 51.11 | 28 | 49.33 | 38 |
| G30 | HYB01# | 1.48 | 22 | 7.87 | 18 | 49.50 | 19 | 47.22 | 36 | 73.05 | 22 |
| G31 | BUT13 | 0.84 | 42 | 6.96 | 28 | 61.00 | 10 | 42.78 | 41 | 50.80 | 37 |
| G32 | BUT14 | 1.89 | 13 | 6.59 | 32 | 32.67 | 36 | 45.56 | 38 | 64.48 | 30 |
| G33 | BUT15 | 1.47 | 25 | 6.99 | 27 | 41.94 | 23 | 55.56 | 20 | 59.87 | 33 |
| G34 | OPV01 | 1.19 | 36 | 5.66 | 37 | 39.33 | 30 | 52.22 | 26 | 45.55 | 39 |
| G35 | HYB02# | 2.24 | 8 | 7.03 | 26 | 41.22 | 24 | 67.78 | 1 | 89.98 | 6 |
| G36 | HYB03 | 1.30 | 29 | 6.74 | 29 | 56.83 | 11 | 67.78 | 1 | 72.37 | 23 |
| G37 | HYB04# | 1.28 | 30 | 5.79 | 36 | 52.06 | 16 | 63.33 | 8 | 66.78 | 25 |
| G38 | HYB05# | 1.26 | 31 | 7.55 | 21 | 67.39 | 4 | 63.33 | 8 | 85.02 | 12 |
| G39 | HYB06# | 1.49 | 20 | 7.08 | 25 | 54.83 | 13 | 64.44 | 7 | 80.61 | 14 |
| G40 | OPV02 | 1.47 | 25 | 6.65 | 31 | 36.50 | 32 | 43.89 | 40 | 54.38 | 34 |
| G41 | HYB07# | 2.24 | 8 | 7.99 | 16 | 39.56 | 28 | 44.44 | 39 | 86.95 | 11 |
| G42 | HYB08# | 1.00 | 40 | 8.40 | 9 | 76.00 | 1 | 50.00 | 33 | 75.42 | 19 |
| | Mean | 1.69 | | 7.28 | | 46.38 | | 55.62 | | 70.85 | |
| | Minimum | 0.84 | | 2.30 | | 9.61 | | 41.11 | | 25.80 | |
| | Maximum | 4.00 | | 9.15 | | 76.00 | | 67.78 | | 95.97 | |
| | LSD | 0.13 | | 0.91 | | 5.17 | | 5.12 | | 8.30 | |
| | CV% | 11.80 | | 19.10 | | 17.00 | | 14.00 | | 17.90 | |

# Commercially available in South Africa; LSD: Least significant difference, CV: Coefficient of variation; Top 10 and bottom 10 ranked genotypes are highlited in green and orange respectively

IBD usually occurs after extended storage. The top four ranking individuals were all goose-neck type fruit, with a very short neck and large seed cavity. A number of genotypes that ranked high for IBD also ranked high for TSS and DMC namely G34, G40, G7, G20, G31 and G19. All genotypes related to G8 ranked very low for IBD (Table 3.8).

TSS and DMC ranged from 5.6 °Brix to 12.1 °Brix and 7.6% to 15.6% respectively. In this study, G7 had the highest ranking for both TSS and DMC. The top 10 genotypes ranked according to TSS were also the top 10 genotypes for DMC. Similarly, the lowest nine genotypes for DMC ranked lower for TSS. All commercial varieties ranked in the lower half for both TSS and DMC, with the exception of G42 and G30 (Table 3.8).

PEN, which is an indication of mesocarp firmness, ranged from 1.8 kg to 3.6 kg, with G3 as the highest ranking genotype. All material related to G3 performed better than average with none of the commercial varieties ranking in the top 10 (Table 3.8).

### 3.5 Discussion

Forty-two different *C. moschata* genotypes were included in this study and evaluated for 15 different characteristics. All characteristics showed substantial variation with highly significant differences. Seven genotypes (G30, G35, G37, G38, G39, G41 and G42) are well-known commercially produced varieties in South Africa. Interestingly, these varieties ranked across the upper and lower spectrums of various plant characteristics, indicating these traits are likely of little economic importance.

Great diversity has been described in *C. moschata* for various characteristics with monetary value, but limited information is available in butternut regarding lesser important traits described in other crops, such as leaf colour. This study confirmed that digital images could be used to accurately quantify canopy colour in *C. moschata*. Leaf colour in *C. moschata* has only been visually rated up to now, according to light-green or dark-green hues (Du *et al.*, 2011), with special reference to presence or absence of silver mottling (Kiramana and Isutsa, 2017). The colour values in this study were based on overall plot averages only and were not aimed specifically on the green background colour. To some extent the quantification of the green component in these values may be skewed by the presence of silver mottling in some varieties. In cereal grasses, primary spectral values for canopy colour obtained from aerial images were used to classify types in different clusters (Constantinescu *et al.,* 2017), while in turf grasses digital images were used to quantify turf colour for objective comparisons (Karcher and Richardson, 2003).

**Table 3.8** Mean values and rankings of total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) evaluated across three environments and two growing seasons

| | | TSS (°Brix) | | DMC (%) | | Fruit a* | | IBD (%) | | PEN (kg) | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Entry | Genotype | Mean | Rank | Mean | Rank | Mean | Rank | Mean | Rank | Mean | Rank |
| G1 | BUT01 | 7.97 | 19 | 10.66 | 16 | 14.99 | 36 | 68.44 | 35 | 2.64 | 21 |
| G2 | BUT02 | 7.78 | 24 | 10.36 | 19 | 20.12 | 10 | 62.08 | 38 | 2.60 | 24 |
| G3 | BUT03 | 9.12 | 9 | 11.82 | 7 | 18.24 | 19 | 79.17 | 28 | 3.57 | 1 |
| G4 | BUT04 | 7.29 | 26 | 9.03 | 34 | 18.00 | 20 | 85.17 | 14 | 2.57 | 26 |
| G5 | BUT05 | 6.75 | 37 | 8.58 | 41 | 11.79 | 40 | 79.51 | 26 | 2.00 | 40 |
| G6 | BUT06 | 10.68 | 2 | 15.34 | 2 | 23.33 | 4 | 84.00 | 17 | 2.46 | 31 |
| G7 | BUT07 | 12.13 | 1 | 15.61 | 1 | 26.65 | 3 | 87.01 | 7 | 3.01 | 4 |
| G8 | BUT08 | 8.31 | 15 | 10.16 | 20 | 27.25 | 2 | 29.89 | 42 | 1.82 | 42 |
| G9 | BUT01xBUT05 | 6.96 | 32 | 9.28 | 28 | 15.15 | 34 | 79.83 | 24 | 2.72 | 16 |
| G10 | BUT02xBUT05 | 6.60 | 39 | 8.87 | 35 | 16.93 | 25 | 74.33 | 34 | 2.67 | 19 |
| G11 | BUT03xBUT05 | 7.15 | 31 | 9.39 | 26 | 16.28 | 29 | 84.56 | 16 | 3.00 | 5 |
| G12 | BUT04xBUT05 | 7.21 | 29 | 9.24 | 29 | 15.86 | 31 | 78.75 | 29 | 2.71 | 17 |
| G13 | BUT01xBUT06 | 8.30 | 16 | 11.04 | 14 | 17.56 | 21 | 85.31 | 13 | 2.89 | 9 |
| G14 | BUT02xBUT06 | 8.11 | 18 | 10.71 | 15 | 19.93 | 13 | 80.64 | 23 | 2.92 | 8 |
| G15 | BUT03xBUT06 | 8.90 | 10 | 11.63 | 8 | 18.31 | 18 | 81.11 | 20 | 3.35 | 2 |
| G16 | BUT04xBUT06 | 8.50 | 13 | 10.50 | 18 | 20.47 | 9 | 85.44 | 12 | 2.95 | 6 |
| G17 | BUT01xBUT07 | 9.19 | 7 | 11.93 | 5 | 19.01 | 15 | 84.67 | 15 | 2.93 | 7 |
| G18 | BUT02xBUT07 | 8.69 | 11 | 11.30 | 11 | 20.10 | 11 | 81.22 | 19 | 2.85 | 11 |
| G19 | BUT03xBUT07 | 9.46 | 4 | 12.03 | 4 | 19.46 | 14 | 86.08 | 10 | 3.23 | 3 |
| G20 | BUT04xBUT07 | 9.19 | 7 | 11.55 | 10 | 21.50 | 6 | 86.78 | 8 | 2.89 | 9 |
| G21 | BUT01xBUT08 | 7.20 | 30 | 9.24 | 29 | 15.57 | 33 | 62.81 | 37 | 2.50 | 30 |
| G22 | BUT02xBUT08 | 6.53 | 40 | 8.63 | 39 | 17.41 | 23 | 56.56 | 40 | 2.58 | 25 |
| G23 | BUT03xBUT08 | 6.94 | 33 | 9.31 | 27 | 16.76 | 27 | 56.22 | 41 | 2.79 | 13 |
| G24 | BUT04xBUT08 | 7.35 | 25 | 9.51 | 24 | 18.73 | 16 | 68.33 | 36 | 2.42 | 32 |
| G25 | BUT09 | 7.79 | 23 | 9.50 | 25 | 5.66 | 42 | 89.85 | 2 | 2.19 | 38 |
| G26 | BUT03xBUT09 | 6.94 | 33 | 8.87 | 35 | 8.97 | 41 | 90.00 | 1 | 2.85 | 11 |
| G27 | BUT06xBUT09 | 8.15 | 17 | 9.98 | 21 | 12.87 | 39 | 88.89 | 3 | 2.69 | 18 |
| G28 | BUT10xBUT11 | 7.25 | 27 | 9.17 | 32 | 14.39 | 37 | 88.11 | 4 | 2.38 | 34 |
| G29 | BUT12 | 7.81 | 22 | 9.97 | 22 | 15.61 | 32 | 77.11 | 31 | 2.25 | 36 |
| G30 | HYB01# | 7.83 | 21 | 10.59 | 17 | 18.66 | 17 | 77.72 | 30 | 2.73 | 14 |
| G31 | BUT13 | 9.35 | 6 | 13.26 | 3 | 27.62 | 1 | 86.56 | 9 | 2.73 | 14 |
| G32 | BUT14 | 7.94 | 20 | 9.94 | 23 | 15.04 | 35 | 86.06 | 11 | 2.67 | 19 |
| G33 | BUT15 | 8.64 | 12 | 11.23 | 13 | 21.73 | 5 | 80.89 | 21 | 2.39 | 33 |
| G34 | OPV01 | 9.45 | 5 | 11.60 | 9 | 16.16 | 30 | 87.14 | 5 | 2.30 | 35 |
| G35 | HYB02# | 5.62 | 42 | 7.57 | 42 | 13.11 | 38 | 60.30 | 39 | 2.19 | 38 |
| G36 | HYB03 | 6.89 | 35 | 9.15 | 33 | 17.56 | 21 | 76.33 | 33 | 2.55 | 28 |
| G37 | HYB04# | 6.53 | 40 | 8.62 | 40 | 20.06 | 12 | 79.58 | 25 | 1.90 | 41 |
| G38 | HYB05# | 6.69 | 38 | 8.79 | 38 | 16.66 | 28 | 79.19 | 27 | 2.54 | 29 |
| G39 | HYB06# | 6.81 | 36 | 8.80 | 37 | 16.78 | 26 | 77.11 | 31 | 2.57 | 26 |
| G40 | OPV02 | 9.68 | 3 | 11.87 | 6 | 21.38 | 7 | 87.07 | 6 | 2.20 | 37 |
| G41 | HYB07# | 7.22 | 28 | 9.24 | 29 | 17.24 | 24 | 82.19 | 18 | 2.63 | 22 |
| G42 | HYB08# | 8.50 | 13 | 11.26 | 12 | 20.97 | 8 | 80.78 | 22 | 2.63 | 22 |
| | Mean | 7.99 | | 10.36 | | 17.85 | | 78.16 | | 2.63 | |
| | Minimum | 5.62 | | 7.57 | | 5.66 | | 29.89 | | 1.82 | |
| | Maximum | 12.13 | | 15.61 | | 27.62 | | 90.00 | | 3.57 | |
| | LSD | 0.54 | | 0.69 | | 1.56 | | 5.92 | | 0.15 | |
| | CV% | 10.30 | | 10.20 | | 13.30 | | 11.60 | | 8.60 | |

# Commercially available in South Africa; LSD: Least significant difference, CV: Coefficient of variation; Top 10 and bottom 10 ranked genotypes are highlited in green and orange respectively

In addition to leaf colour, CHL in *C. moschata* has been shown to be influenced by plant nutritional status because of differences in growing media (Okonwu *et al.,* 2018). The CHL measurements for all genotypes in this study ranged from 30.2 to 48.3, which are in agreement with another study (Lin *et al*., 2020). These authors reported CHL measurements ranging from 28.1 to 53.4 and indicated significant differences between the *C. moschata* and *C. maxima* cultivars, but due to the limited number of cultivars included, no differences in CHL within species were noticed. The CHL results fall within the range measured in other crops, for example CHL measurements in wheat ranged from 30.8 to 47.3 under non-stress conditions (Azadi *et al.,* 2011) and in durum wheat it ranged from 42.3 to 71.5 under different irrigations (Karimizadeh *et al.,* 2011). In tropical maize, CHL ranged from 14.2 to 53.3 (Betran *et al.,* 2003). In a separate maize study, an average CHL content of 56.4 was measured under full-irrigated conditions and 44.1 under drought conditions (Khayatnezhad *et al.,* 2011).

Lin *et al.* (2020) demonstrated that stable CHL levels could be used to identify cucurbit genotypes tolerant to water-logged conditions. In maize (Betran *et al.,* 2003), barley (Rong-hua *et al.,* 2006), cotton (Karademir *et al.,* 2009) and wheat (Azadi *et al.,* 2011) varieties tolerant to abiotic stresses showed smaller fluctuations in CHL levels compared to less tolerant varieties. Differences in CHL between genotypes had a significantly positive correlation with grain yield in maize (Ghimire *et al.,* 2015) and yield components in cotton (Karademir *et al.,* 2008). This study showed significant differences among genotypes within *C. moschata,* which might have positive associations with yield components, or tolerance to unfavourable growing conditions.

Significant differences in leaf size have been described among inter- and intra-specific *Cucurbita* crosses, but no significant differences were observed between the two *C. moschata* genotypes studied by El-Tahawey *et al.* (2015). Little additional information is available concerning differences in leaf morphology. This study showed significant differences within butternut genotypes with regard to LW as well as PL. Diverse genotypes exhibited different combinations of characteristics where smaller and larger leaves were associated with both shorter and/or longer petioles. This allows unique genotypes to position their leaves differently for maximum light absorption. In other crops such as maize (Khayatnezhad *et al*., 2011) and rice (Liu *et al*., 2019) photosynthetic efficiency was influenced by CHL.

The current study demonstrated that butternut plant morphological characteristics *per se* may not have monetary value, but the vast quantifiable diversity present sets a scene within which the possibility for correlations with economically important traits can be investigated. These possible correlations could have a significant impact on butternut breeding. Even though these

characteristics may not be linked to specific morphological traits included in this study, they could still have underlying benefits with regard to tolerances to biotic and abiotic stresses.

Since varietal selection for specific market segments is driven by fruit size, AFM is a good indicator of a potentially appropriate market segment. Yield is usually the product of FN and AFM and since yield is dependent on the photosynthetic potential of a plant, AFM is negatively correlated with FN (Tamilselvi *et al.,* 2012; Naik *et al.,* 2015). In addition, yield is usually lower in small-fruited varieties, forcing growers to plant varieties with a lower yield potential so as to meet specific market requirements with regard to fruit sizes. Similar patterns were noticed in this study. Due to limited quality control by chain stores and the end consumer lacking education with regard to internal fruit quality, wholesale growers have little incentive to plant a variety, which has the best quality for eating. Profitability, therefore, is driven by production of maximum fresh weight, independent of internal quality.

As part of the quality assessments in this study, TSS values measured ranged from 5.6 °Brix to 12.1 °Brix. Results are in agreement with other studies where TSS were reported to be 12.1 °Brix in *C. moschata* landrace pumpkin in Brazil (Carvalho *et al.,* 2014) and 9.6 °Brix and 11.0 °Brix in two different cultivars in China (Abbas *et al.,* 2020). It is also in agreement with various other reports where TSS ranged from 7.8 °Brix to 11.7 °Brix (Abdein *et al.*, 2017), 10.2 °Brix to 11.5 °Brix (Noseworthy and Loy, 2008) and 8.2 °Brix to 11.0 °Brix (Akter *et al.,* 2013). A separate study (Carvalho *et al*., 2015) measured TSS ranging from 5.0 °Brix to 17.2 °Brix where the range was much higher than in the current study. The highest value for a commercial variety in the current study was 8.5 °Brix (G42), with all other commercial material ranking below average. From this result, it is clear that TSS could be significantly improved with the current variation available in South African material.

A similar scenario was observed for DMC where data in the current study ranged from 7.6% to 15.6%, with all commercial material (with the exception of G30 and G42) ranking below average. These values are in the bottom range of the DMC results cited in literature, which included 14.7% (Carvalho *et al.,* 2014), 21.1% and 12.9% in two different cultivars (Abbas *et al.,* 2020) and 19.9% in a *C. maxima* variety, Justynka (Sojak *et al.,* 2014). Noseworthy and Loy (2008) found DMC to range from 17.0% to 22.5%, while Akter *et al.* (2013) measured DMC ranging from 6.8% to 17.9%.

Although the ranges with regard to DMC and TSS in this study fit within the variation described in literature, much wider variation is available within the species. A similar trend has been noticed for AFM, where literature refers to AFM ranging from 1.5 kg to 7.8 kg (Carvalho *et al.,*

2015), 0.5 kg to 10.0 kg (Gamboa *et al.,* 2016), 0.7 kg to 7.9 kg (Nagar *et al.,* 2017) and 0.4 kg to 1.3 kg (Noseworthy and Loy, 2008). These value ranges are much wider than the 0.8 kg to 4.0 kg measured in the current study. Based on AFM most genotypes included in this study were smaller than 3.0 kg and will be accepted in the South African market. However, large-fruited genotypes not meeting this requirement may have other morpho-agronomic characteristics which could benefit a South African breeding programme.

With regard to germplasm currently available, genotypes G35 and G41 are the standard commercial varieties in the processing market segment. In this study, both had an AFM of 2.2 kg. G9 and G10 had significantly higher yields than G41, but they were similar to G35 and G11. Although uniformity was poorer in G9, G10 and G11 compared to that in G35, all were significantly more uniform than G41. Internal fruit quality of those crosses ranked higher than G35 across all internal quality characteristics. Although G41 had significantly improved internal colour compared to G9 and with less IBD compared to G10, the higher yields of G9 and G10 placed these hybrids in a more desirable position overall. G11 had a similar yield to G41, but it was more uniform with better internal quality.

Although G35 is one of the leading butternut varieties internationally, it ranked very low for Fruit a* with a value of 13.1. The Fruit a* range (5.7 to 27.6) in this study fell within the previously reported ranges of 14.9 to 26.2 (Francis, 1962), -4.9 to 14.8 (Itle and Kabelka, 2009) and 5.7 to 40.3 (Seroczynska *et al.,* 2006) with higher values associated with darker orange flesh colour. Even though a low value is acceptable to the South African and international consumer, this shows the huge potential to improve flesh colour as a quality characteristic.

Genotypes in the fresh market segment (AFM ranging between 1.3 kg and 2.0 kg), G39 and G30, had an AFM of 1.5 kg with similar DMY and yield. Genotypes G16, G17, G24 and G18, which fit into the same market segment, all had significantly higher or similar DMY, fresh yields and similar or improved TSS, DMC and flesh colour compared to G39. G24 showed more IBD but this would only be a concern when fruit is destined to be stored for extended periods. The overall internal quality of G18 was exceptional, with significantly greater uniformity than G30, although not as uniform as G39.

Genotypes in the small-fruited market segment (AFM smaller than 1.3 kg), G38, G13, G15 and G14, were among the highest yielders, with no significant differences for yield among them. However, G38 had significantly lower DMY (except for G14), TSS and DMC compared to the others. Uniformity of G38 and G15 was significantly better than the other genotypes. The best

performer was G15, which demonstrated the highest overall internal quality without sacrificing yield.

Internal quality is mostly determined by quantifying TSS, DMC and flesh colour, which could be time consuming. PEN as a measurement of flesh firmness is not a well-known characteristic in the industry, but showed huge variation in this study. Since PEN is an easily measured trait, associations with economically important characteristics could be of value in the breeding of butternuts.

### 3.6 Conclusions

All morpho-agronomic and internal fruit quality characteristics studied demonstrated high phenotypic variance, which would permit improvement through phenotypic selection. With the exception of Fruit a*, all characteristics measured showed differences between locations; however, all characteristics, including Fruit a*, demonstrated differential ranking of the genotypes across locations, indicating genotype x environment interactions. The standard commercial varieties were confirmed as suitable choices for commercial growers, although G9, G24 and G15 all achieved high levels of internal quality, yet without sacrificing yield. There is the potential for uniformity to be a little lower, but it remains sufficiently similar to other commercial material and is, therefore, acceptable.

Preceding the commercial release of new material, the stability of the relevant genotypes across environments has to be confirmed, and this is covered in Chapter 4. Chapter 5 will indicate in more detail direct and indirect effects among all characteristics measured. In the current study, hybrid genotypes outperformed their related parental lines for most of the yield and yield component characteristics, which strongly suggested the presence of heterosis. Some parental lines were also better represented among the top-ranking hybrids in terms of specific characteristics, suggesting superior general combining ability. Both combining ability and heterosis were determined and discussed in Chapter 6.

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# CHAPTER 4

# STABILITY ANALYSIS OF BUTTERNUT FOR SELECTED MORPHO-AGRONOMIC AND INTERNAL FRUIT QUALITY CHARACTERISTICS


## 4.1 Abstract

Multi-location trials are vital for improvement programmes to identify high yielding genotypes adapted to a wide range of environments. The aim of this study was to confirm the combination of high performance and stability for both morpho-agronomic and internal fruit quality characteristics in desirable butternut genotypes across different environments. In total 42 genotypes, including hybrids, parental lines and open-pollinated varieties, were evaluated across three locations and two seasons. The data was analysed as six individual environments. The additive main effect and multiplicative interaction analysis of variance indicated significant interactions for average fruit mass, fruit number, yield, dry matter content, green-red colour contribution in the fruit mesocarp and total soluble solids, which confirmed differential response of genotypes across environments. Fruit mass, fruit number and yield displayed much larger differences across environments, compared to fruit quality characteristics, which seemed to be more stable across environments. Based on multiple stability measures, genotypes were classified as stable or unstable for all listed variables. Genotypes G11 and G13 were identified as the most stable for the processing and small-fruited market segments, while G16 and G17 were the most desirable for the fresh market segment. No variety displayed high performance and stability for all characteristics, which still leaves scope for improvement in butternut varieties.



***Keywords*:** AMMI analysis, genotype x environment interaction, GGE biplot analysis, mega-environments, stability


## 4.2 Introduction

The increasing popularity of butternut squash (*Cucurbita moschata)* in South Africa can be attributed to its long shelf life and high nutritional value with regard to phenolic compounds, minerals, vitamins, proteins and carbohydrates (Enneb *et al*., 2020). In many developing countries, including South Africa, butternut plays an important role in food security. Although great diversity is available in the species, the number of butternut varieties available in South Africa is fairly limited. Variety selection for specific market segments is based mostly on a specific fruit size requirement and on yield performance. Although locally developed material compares well with global standards for both yield and internal quality, selection focus on stability and adaptability of material across a wide range of environments is still mostly absent.

Cultivar performance in terms of quantitative characteristics is determined by the genotype, environmental conditions and cultural practices. Performance is a direct result of a genotype's ability to adapt to the surrounding environment (Zakir, 2018). In most genotype screening trials, analysis of variance is routinely used to partition variation into genotype and environment main effects as well as genotype x environment (G x E) interaction. Many of these include multi-location trials conducted in order to study genotypic responses across diverse environments. Significant G x E interactions have been observed in various vegetable crops, including cassava (Peprah *et al.,* 2016), cucumber (Iwo and Odor, 2017), pepper (Barchenger *et al.,* 2018), pickling cucumber (Dia *et al.,* 2018) and tomato (Djidonou *et al.,* 2020), which permitted distinction between stable and unstable; and ultimately identifying the most desirable genotypes. A significant G x E interaction exists when inconsistent rankings are obtained from different locations or seasons, and is the preamble to stability analyses that will identify stable genotypes giving high performance across diverse environments (Djidonou *et al.,* 2020). Higher stability has been accompanied by decreased yield (Dia *et al.,* 2016); therefore, it is important to confirm both performance and stability of new genotypes prior to commercialisation of material.

A number of *C. moschata* studies focused on genotypic diversity (Kumar *et al.,* 2011; Gomes *et al.,* 2020) and inheritance (Gwanama *et al.,* 2001; Pandey *et al.,* 2010; Akter *et al.,* 2013; Nisha and Veeraragavathatham, 2014; Rana *et al.,* 2015; Tamilselvi *et al.,* 2015; Ahmed *et al.,* 2017; Mohsin *et al.,* 2017; Restrepo *et al.,* 2018; Restrepo-Salazar *et al.,* 2019). However, all were conducted in single environments, and subsequently with no reference to environmental variation or G x E interaction. Only a few cited studies were conducted across multiple locations to identify significant environmental variation. Abdein *et al.* (2017) for example demonstrated significant G x E interaction with regards to seed weight of *C. moschata,* while Valdes-Restrepo *et al*. (2013) mentioned stable dry matter content, but unstable fruit production across favourable and unfavourable conditions.

Various statistical methods are available for stability analysis of which two multivariate models have been the most commonly used in recent years. These are additive main effects and multiplicative interaction (AMMI) and genotype main effect plus genotype x environment interaction (GGE) and both are based on principal component analysis (PCA) in order to explain the stability of genotypes across environments using graphical displays (Dia *et al.,* 2016).

Various genotypes were identified in Chapter 3 as high performers for characteristics including average fruit mass, dry matter yield, dry matter content, fruit number, uniformity, total soluble

sugars and flesh colour that demonstrate the possibility of improving internal quality without sacrificing yield. The aim of this study was to confirm the possibility of combined high performance and stability for various yield and quality characteristics in desirable butternut genotypes. The objectives were to: (i) confirm the combination of high performance and stability for both morpho-agronomic and internal fruit quality characteristics across different environments; (ii) demonstrate the application of AMMI and GGE biplots; and (iii) identify the most desirable genotypes for diverse market segments while taking all characteristics into account.

## 4.3 Materials and methods

### 4.3.1 Site description, trial design and plant material

Forty-two genotypes were evaluated during the summers of 2018/2019 and 2019/2020 in three locations across South Africa viz. Jacobsdal, Kaalfontein and Oudtshoorn. Each location within a specific season represented a unique environment (E) resulting in six different environments (E1 to E6). E1 and E2 represented Jacobsdal, E3 and E4 represented Kaalfontein and E5 and E6 represented Oudtshoorn for each of the two seasons, respectively. Refer to Chapter 3, Sections 3.3.1 to 3.3.2 for detailed descriptions regarding the sites, trial design and management, and plant material used in this study.

### 4.3.2 Data collection and statistical analysis

Refer to Chapter 3, Section 3.3.3 for detailed descriptions regarding the collection of data on morpho-agronomic and internal fruit quality characteristics used in this study. Earlier interpretation of analysis of variance (ANOVA) in Chapter 3 displayed significant G x E interactions in most characteristics of which the most important included yield, average fruit mass (AFM) per plot, fruit number (FN) per plot, total soluble solids (TSS), dry matter content (DMC) and green-red colour contribution in the fruit mesocarp (Fruit a*). For this study, data on these six characteristics were, therefore, subjected to both AMMI and GGE biplot analyses using Genstat® software (VSN International, 2017).

<u>AMMI analysis</u>

AMMI analysis is an ANOVA that partitions the variance into genotype, environment and G x E interaction effects, in combination with a multiplication effect analysis, which in turn partitions the G x E interaction effect into various interaction principal component axes (IPCA). These can then be tested for significance (Shafii and Price, 1992; Farshadfar *et al.,* 2011). The AMMI model is described by the equation:

$$Y_{\mathrm{ij}} = \mu + \mathrm{g}_i + e_j + \sum_{n=1}^{N} \lambda_k \gamma_{ik} \delta_{jk} + \varepsilon_{ij}$$

Where $Y_{\mathrm{ij}}$ is the yield of $i$-th genotype in the $j$-th environment; μ is the grand mean; $g_i$ and $e_j$ are the deviations of genotype and environment from the grand mean, respectively. $\lambda_k$ is the eigenvalue of the PCA for axis $k$; $y_{ik}$ and $\sigma_{jk}$ are the genotype and environment principal component (PC) scores for axis $k$; $N$ is the number of principal components in the AMMI model; $\varepsilon_{ij}$ is the residual term. Genotype and environment PCA scores are expressed as unit vector times the square root of $\lambda_k$ (genotype PCA score = $\lambda_k^{0.5}\ y_{ik}$, environment PCA score = $\lambda_k^{0.5}\ \sigma_{jk}$) (Zobel *et al.,* 1988). The genotype main effects were plotted against the IPCA to display the data graphically.

The AMMI model 1 biplot illustrates the main effects and the first interaction principle component axis (IPCA1) on the x-axis and y-axis, respectively. Those genotypes that were least interactive with the environment have a lower IPCA1 score and indicate general adaptation across environments. Genotypes arranged along a vertical line share similar mean performances, while those arranged along a horizontal line have similar interaction patterns (Barchenger *et al.,* 2018). Genotypes and environments with similar IPCA1 scores and signs, indicate specific adaptation of those genotypes to those particular environments (Dia *et al.,* 2016). Genotypes in close proximity within the biplot indicate similarity of response and identify potential genotype trends across similar environments (Shafii and Price, 1992). Genotypes with opposite signs represent crossover interactions and are adapted to different specific environments (Farshadfar *et al.,* 2011). Environments with a large IPCA score have a greater influence on genotype means, compared to those with smaller values (Barchenger *et al.,* 2018). The position where the vertical axis intersects the horizontal axis represented the mean across all environments. The high performance genotypes are positioned to the far right of the biplot and the more stable ones closer to the x-axis.

In instances where data points are concentrated in certain areas on the graph, it can be difficult to interpret the data. AMMI stability value (ASV) was proposed to describe stability of genotypes (Purchase *et al.,* 2000) making it easier to rank genotypes for stability. Although it has not previously been used in butternut, it showed significant correlation with other measures of stability in wheat (Purchase *et al.,* 2000; Farshadfar *et al.,* 2011) and sugarcane (Tena *et al.,* 2019). The ASV scores were calculated using the equation:

$$ASV = \sqrt{\left[\frac{IPCA1_{sum\ of\ square}}{IPCA2_{sum\ of\ square}}(IPCA_{score})\right]^2 + (IPCA2_{score})^2}$$

ASV is defined as the distance from the biplot origin to a genotype marker on a two-dimensional scatter plot of IPCA1 scores against the second interaction principle component axis (IPCA2) scores. Since the IPCA1 contributes a larger fraction to the interaction sum of squares it is weighted by the proportional difference between IPCA1 sum of squares and IPCA2 sum of squares. The distance from zero is then determined using the theorem of Pythagoras (Purchase *et al.,* 2000). The smaller the ASV score, the more stable the genotype is across environments. Since stability is only of value in high yielding genotypes, yield stability index (YSI) was calculated using the equation:

$$YSI = RASV + RY$$

Where RASV is the rank of the ASV and RY the mean genotype performance rank across environments (Farshadfar *et al.,* 2011). YSI incorporates both mean performance and stability in a single criterion, where a lower value represents desirable genotypes with high stability and performance. In this study, YSI was not only applied to yield, but also to other characteristics evaluated for stability.

GGE biplot analysis

In addition to AMMI analysis, the GGE biplot method as proposed by Yan (2002) was used to present genotype and G x E interaction patterns graphically. The GGE scatter plot was used to identify mega-environments and their associated top performing genotypes. The first and second PC scores of the genotypes were used to construct a GGE biplot. Both positive and negative PC scores indicate divergent responses in performance among genotypes across environments, due to G x E interactions. The extreme genotypes located furthest away from the biplot origin in all possible directions are selected in such a way that the resulting polygon includes all genotype markers. Rays from the biplot origin perpendicular to the polygon sides divide the biplot into a number of sectors (Yan *et al.,* 2007). Environments appearing in the same sector are collectively referred to as a mega-environment, with the vertex genotype in that sector being the best performer in at least one of the relevant environments, and one of the best performing genotypes in other environments (Tena *et al.,* 2019). In biplots with more than one mega-environment, different sectors have different genotypes as top performers, indicating crossover G x E interaction patterns. Genotypes in a sector with no environments

featuring are known to be poor performers across all environments. Genotypes close to the biplot origin are less responsive to environments and are therefore known to be more stable.

The GGE ranking plots were used to rank genotypes according to performance and stability. The line passing through the biplot origin and the average environment coordinate (AEC) is known as the average environment axis (AEA). The arrow shown on the AEA indicates the direction of higher-trait performance of the genotypes. Projections of genotype markers onto the AEA show the mean performance of specific genotypes. Therefore, genotype performances are ranked along the ordinate. The distance from the genotype marker to the AEA is an indication of a genotype's stability, where near-zero values represent the most stable genotypes (Shim *et al.,* 2015).

The comparison biplots were used to identify the most desirable genotype for each characteristic. Stability is only meaningful when considered in association with mean performance; however, stability can also be associated with poor performance. Stability in plant breeding is therefore only of value when associated with high mean performance (Yan and Tinker, 2006). In the comparison biplot, an ideal genotype is represented by an arrow tip in the inner circle and defined as the longest vector length of the high yielding genotypes with zero G x E interaction (Tena *et al.,* 2019). The closer a genotype marker is to the ideal genotype position, the more desirable it will be.

### 4.4 Results

Based on the combined ANOVA presented in Chapter 3, all six characteristics included in this chapter showed significant differences with regard to genotype, location, season and various interactions among the main effects. This confirmed that experiments were carried out with different genotypes under different environmental conditions. The significant interaction effect indicates that various genotypes were influenced differently across seasons and locations, which added to the phenotypic variation observed. This variation was useful for G x E interaction studies in order to evaluate various traits for phenotypic stability across different environments.

#### 4.4.1 Genotype x environment interaction: AMMI analysis of variance

An ANOVA using the AMMI model confirmed highly significant ($P \leq 0.001$) genotype, environment and G x E interaction effects (Table 4.1) for AFM, FN, yield, DMC, Fruit a* and TSS. In the case of all, except yield, the genotypes accounted for over 50% of the variation within the total sum of squares. Yield was the only trait for which the environment was the main contributor to the total variation (51%). Around 25% of the total variation in AFM and FN

was attributed to the environment main effect. In all cases, the G x E interaction effect contributed the least to the total variation, ranging between 9.8% (AFM) and 15.0% (TSS).

The G x E interaction effect was further partitioned into the first two IPCA, which together explained a total of 75.2% and 69.5% for AFM and yield respectively (Table 4.1). IPCA1 was particularly important and explained around 50% of the interaction variation in AFM and yield. The first (36.1%) and second (31.2%) IPCA axes made similar contributions to the G x E interaction variation in Fruit a*, but explained only 67.3% of the interaction variation. For FN, DMC and TSS, IPCA2 explained less than two thirds of the interaction variation.

### 4.4.2 AMMI model 1 biplot analysis

With regard to AFM, G25 produced the largest fruit but it was less stable than G27, which had the second largest fruit (Figure 4.1A). G12 and G5 were highly unstable. A large number of genotypes were concentrated around the biplot origin, all representing stable genotypes with fruit sizes between 1.5 kg and 2.0 kg. G42 was the best performer but the least stable genotype for FN (Figure 4.1B). With regard to yield, G2, G6, G7, G8 and G25 were all below average and unstable (Figure 4.1C). Genotype G10 appeared to be stable together with a high yield. The top ranking genotypes for TSS were G6 and G7 but they were both unstable (Figure 4.1D). Although G40 and G42 had opposing IPCA signs, both were highly unstable. Genotype G17 demonstrated stability and was under the top performers for TTS. For DMC, G6, G34, G39, G40 and G42 were highly unstable (Figure 4.1E). Genotype G25 was not only the least stable genotype in terms of Fruit a*, it also had the lowest performance (Figure 4.1F). For yield, environments E1 and E5 had the highest overall averages, but E1 exerted the greatest interactive force (Figure 4.1C). In this study where a large number of genotypes clustered together on a biplot, it is essential to quantify stability in order to rank genotypes according to stability, and subsequently stability and performance.

The ASV and YSI for the six characteristics evaluated for each genotype are summarised in Tables 4.2 and 4.3. Stable genotypes are represented by lower ASV scores, while lower YSI scores represent more desirable genotypes. The top 10 and bottom 10 genotypes for each characteristic were indicated in green and orange respectively. The most stable genotype for AFM was G24, which was also the second most desirable according to the YSI ranking. Genotype G17 was most desirable for AFM. For FN and yield the most stable genotypes were G18 and G20, but the most desirable were G13 and G10, respectively. For DMC, the most stable and desirable genotype was G16. Genotype G20 was the most desirable for both Fruit a* and TSS. No genotype emerged as being consistently stable or desirable for all characteristics evaluated.

**Table 4.1** AMMI analysis of variance for average fruit mass (AFM), fruit number (FN), yield, dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*) and total soluble solids (TSS) across six environments

| | | AFM | | FN | | Yield | | DMC | | Fruit a* | | TSS | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | df | MS | % Var | MS | % Var | MS | % Var | MS | % Var | MS | % Var | MS | % Var |
| Total | 755 | 0.61 | | 423 | | 1030 | | 4.80 | | 26.40 | | 2.77 | |
| Treatments | 251 | 1.77 *** | 95.62 | 1146 *** | 89.97 | 2772 *** | 89.43 | 12.11 *** | 83.76 | 67.88 *** | 85.48 | 6.96 *** | 83.71 |
| Genotypes | 41 | 7.07 *** | 62.46 | 4116 *** | 52.79 | 5369 *** | 28.29 | 51.79 *** | 58.53 | 327.23 *** | 67.31 | 28.59 *** | 56.16 |
| Environments | 5 | 21.72 *** | 23.42 | 16919 *** | 26.46 | 79742 *** | 51.25 | 93.30 *** | 12.88 | 237.79 *** | 5.97 | 52.55 *** | 12.59 |
| Block | 12 | 0.15 *** | 0.37 | 183 *** | 0.69 | 755 *** | 1.16 | 9.54 *** | 3.14 | 35.50 *** | 2.14 | 1.70 ** | 0.98 |
| Interactions | 205 | 0.22 *** | 9.75 | 167 *** | 10.72 | 375 *** | 9.88 | 2.19 *** | 12.35 | 11.86 *** | 12.20 | 1.52 *** | 14.96 |
| IPCA 1 | 45 | 0.49 *** | | 273 *** | | 862 *** | | 3.67 *** | | 19.52 *** | | 2.42 *** | |
| IPCA 2 | 43 | 0.29 *** | | 174 *** | | 340 *** | | 2.61 *** | | 17.64 *** | | 1.57 *** | |
| Residuals | 117 | 0.10 *** | | 124 *** | | 201 * | | 1.46 ** | | 6.79 * | | 1.16 *** | |
| Error | 492 | 0.04 | | 61 | | 149 | | 0.96 | | 5.02 | | 0.65 | |
| % G x E due to IPCA1 | | 48.23 | | 35.92 | | 50.44 | | 36.83 | | 36.10 | | 34.90 | |
| % G x E due to IPCA2 | | 26.99 | | 21.79 | | 19.01 | | 25.00 | | 31.21 | | 21.68 | |

Treatments: genotypes, environments and interactions combined, *P≤0.05, **P≤0.01, ***P≤0.001, df: degrees of freedom, MS: Mean squares, % Var: percentage of total sum of squares, IPCA: interaction principal component analysis axis, G x E: genotype environment interaction

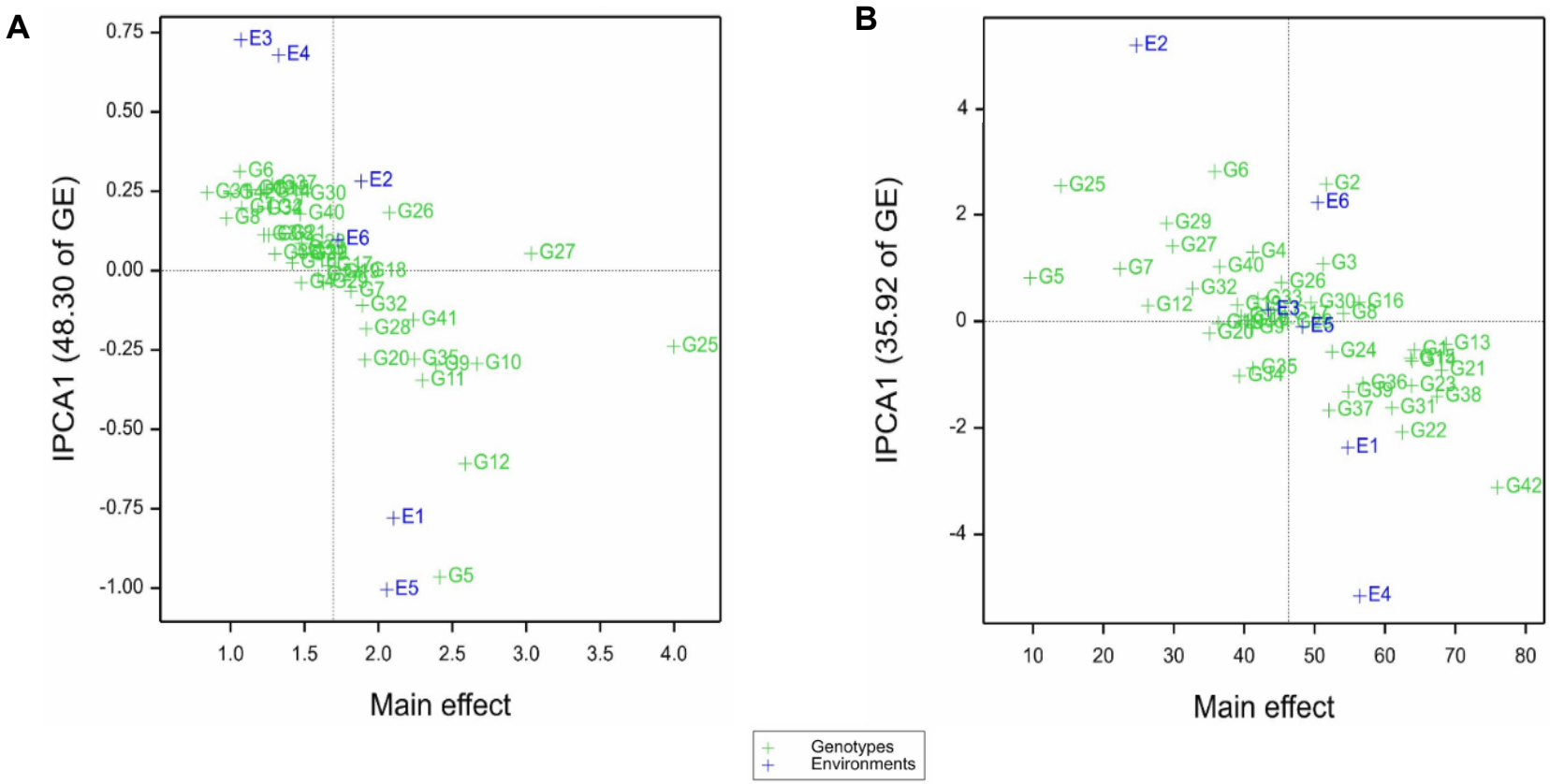


**Figure 4.1** AMMI model 1 biplots showing the main and first interaction principal components (IPCA1) for selected morpho-agronomic and internal quality characteristics. A: average fruit mass (AFM), B: fruit number (FN), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020

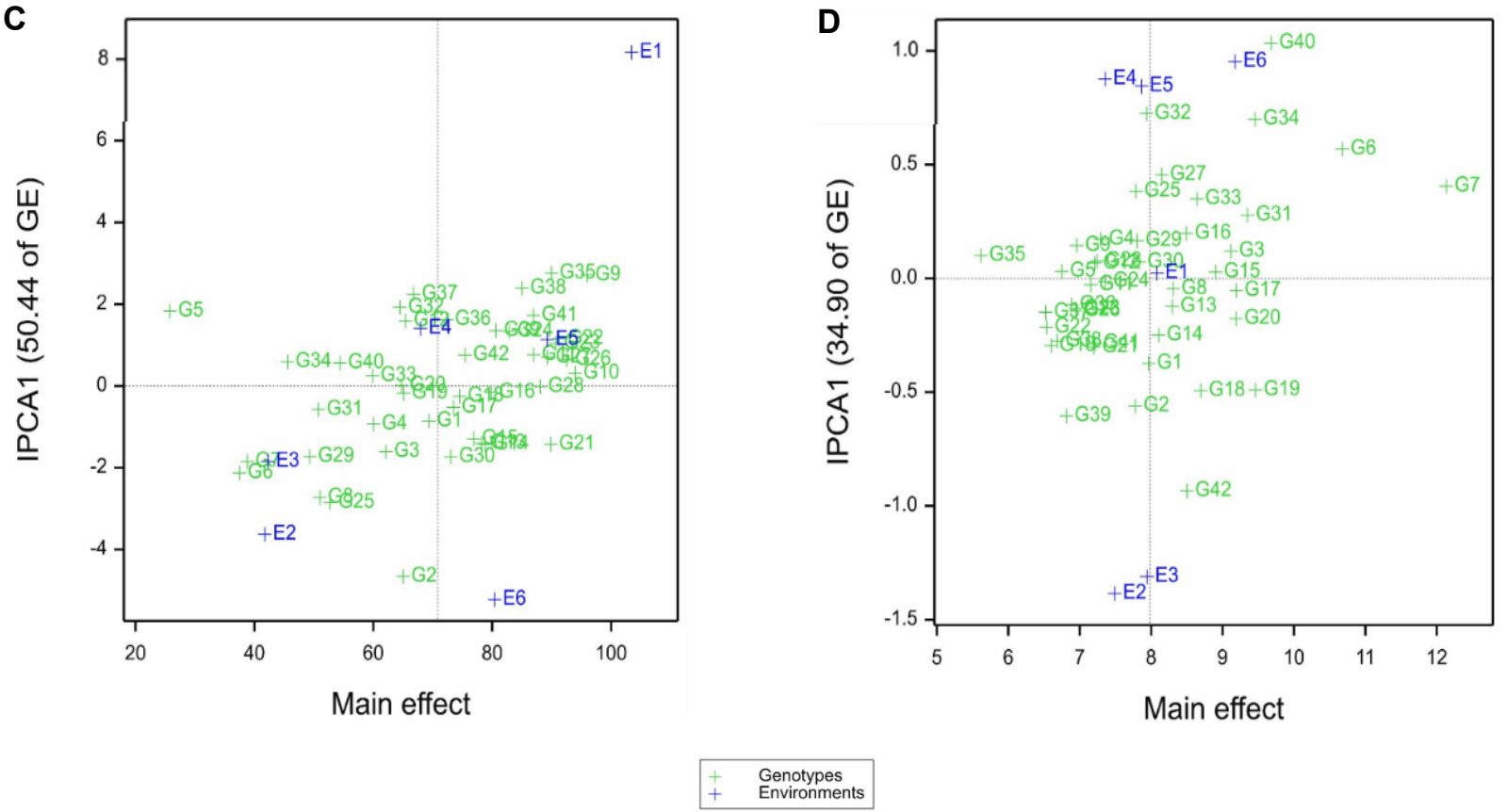


**Figure 4.1** AMMI model 1 biplots showing the main and first interaction principal components (IPCA1) for selected morpho-agronomic and internal quality characteristics. C: yield, D: total soluble solids (TSS), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020

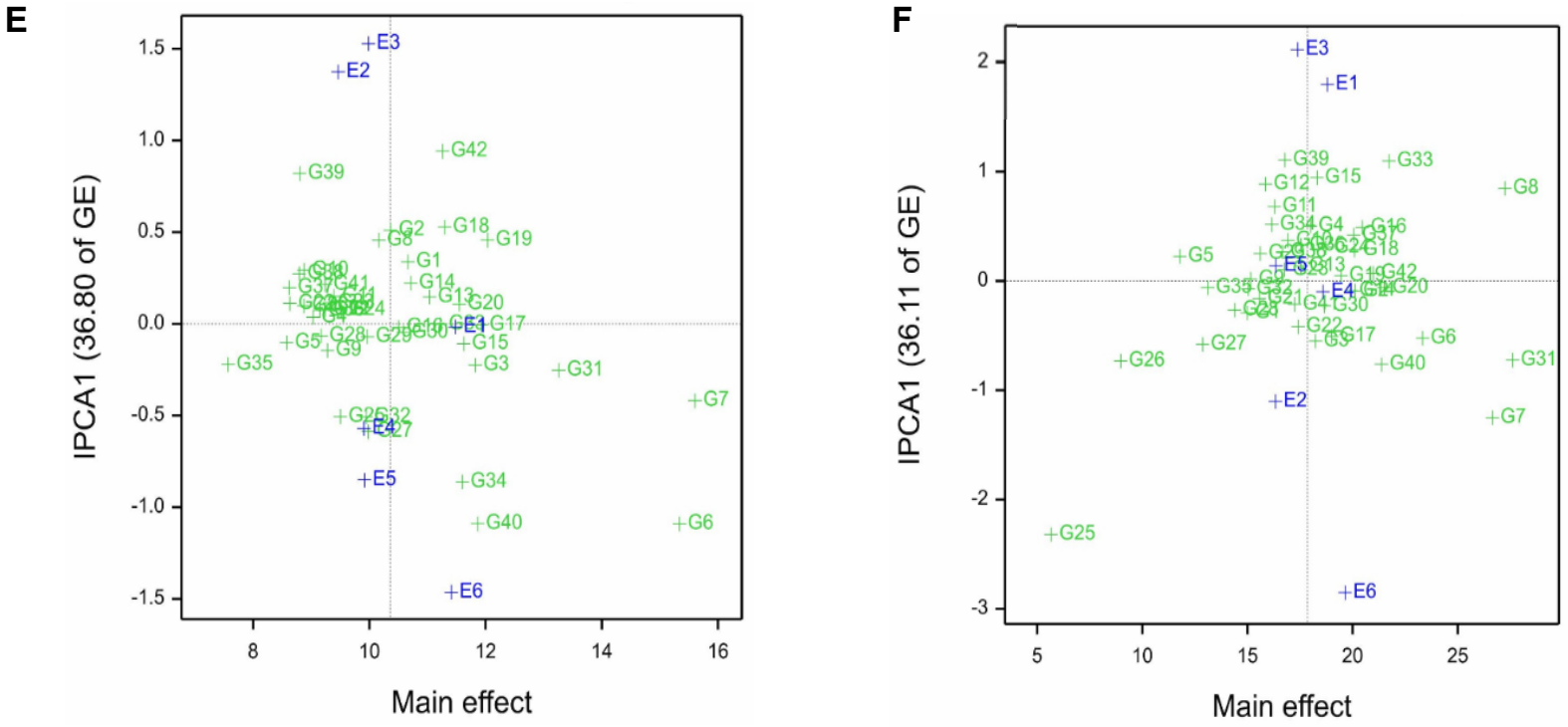


**Figure 4.1** AMMI model 1 biplots showing the main and first interaction principal components (IPCA1) for selected morpho-agronomic and internal quality characteristics. E: dry matter content (DMC), F: green-red colour contribution in fruit mesocarp (Fruit a*), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020

**Table 4.2** AMMI stability value (ASV) and yield stability index (YSI) for average fruit mass (AFM), fruit number (FN) and yield for 42 genotypes

| | | AFM | | | | FN | | | | Yield | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Entry | Genotype | ASV | RASV | YSI | RYSI | ASV | RASV | YSI | RYSI | ASV | RASV | YSI | RYSI |
| G1 | BUT01 | 0.40 | 26 | 64 | 37 | 1.40 | 18 | 23 | 5 | 2.46 | 15 | 39 | 19 |
| G2 | BUT02 | 0.39 | 24 | 55 | 32 | 4.52 | 40 | 57 | 33 | 12.38 | 42 | 70 | 37 |
| G3 | BUT03 | 0.21 | 13 | 48 | 30 | 1.88 | 24 | 42 | 19 | 4.58 | 28 | 59 | 33 |
| G4 | BUT04 | 0.25 | 17 | 41 | 19 | 2.14 | 27 | 51 | 29 | 3.06 | 17 | 49 | 27 |
| G5 | BUT05 | 1.84 | 42 | 47 | 29 | 1.42 | 19 | 61 | 36 | 5.38 | 34 | 76 | 40 |
| G6 | BUT06 | 0.63 | 39 | 78 | 42 | 5.03 | 41 | 75 | 41 | 5.87 | 35 | 76 | 40 |
| G7 | BUT07 | 0.23 | 15 | 30 | 10 | 1.79 | 23 | 63 | 37 | 4.90 | 32 | 72 | 38 |
| G8 | BUT08 | 0.31 | 18 | 59 | 34 | 2.52 | 32 | 46 | 26 | 7.47 | 39 | 75 | 39 |
| G9 | BUT01xBUT05 | 0.54 | 35 | 41 | 19 | 0.23 | 2 | 28 | 9 | 7.20 | 38 | 39 | 19 |
| G10 | BUT02xBUT05 | 0.56 | 37 | 40 | 18 | 1.26 | 12 | 45 | 25 | 1.43 | 5 | 7 | 1 |
| G11 | BUT03xBUT05 | 0.62 | 38 | 45 | 25 | 1.39 | 16 | 44 | 23 | 2.33 | 14 | 24 | 6 |
| G12 | BUT04xBUT05 | 1.10 | 41 | 45 | 25 | 0.86 | 7 | 46 | 26 | 4.28 | 25 | 51 | 31 |
| G13 | BUT01xBUT06 | 0.48 | 32 | 69 | 40 | 0.92 | 8 | 10 | 1 | 4.55 | 27 | 44 | 24 |
| G14 | BUT02xBUT06 | 0.46 | 29 | 62 | 35 | 1.33 | 14 | 20 | 4 | 4.18 | 24 | 40 | 21 |
| G15 | BUT03xBUT06 | 0.46 | 30 | 64 | 37 | 1.14 | 9 | 17 | 3 | 3.54 | 20 | 38 | 18 |
| G16 | BUT04xBUT06 | 0.13 | 8 | 35 | 15 | 0.59 | 4 | 16 | 2 | 1.28 | 4 | 19 | 4 |
| G17 | BUT01xBUT07 | 0.05 | 2 | 19 | 1 | 0.58 | 3 | 24 | 6 | 1.46 | 6 | 27 | 9 |
| G18 | BUT02xBUT07 | 0.11 | 7 | 21 | 3 | 0.19 | 1 | 28 | 9 | 2.20 | 13 | 33 | 14 |
| G19 | BUT03xBUT07 | 0.20 | 12 | 28 | 9 | 0.62 | 5 | 36 | 15 | 0.97 | 3 | 30 | 10 |
| G20 | BUT04xBUT07 | 0.50 | 34 | 46 | 27 | 1.39 | 17 | 52 | 30 | 0.05 | 1 | 30 | 10 |
| G21 | BUT01xBUT08 | 0.24 | 16 | 44 | 22 | 1.60 | 22 | 25 | 7 | 3.83 | 23 | 30 | 10 |
| G22 | BUT02xBUT08 | 0.09 | 5 | 25 | 7 | 3.50 | 38 | 47 | 28 | 3.17 | 18 | 22 | 5 |
| G23 | BUT03xBUT08 | 0.20 | 10 | 33 | 12 | 2.05 | 26 | 33 | 12 | 3.54 | 19 | 24 | 6 |
| G24 | BUT04xBUT08 | 0.04 | 1 | 20 | 2 | 1.15 | 10 | 25 | 7 | 3.80 | 22 | 35 | 15 |
| G25 | BUT09 | 0.96 | 40 | 41 | 19 | 4.30 | 39 | 80 | 42 | 7.85 | 41 | 76 | 40 |
| G26 | BUT03xBUT09 | 0.35 | 23 | 33 | 12 | 1.55 | 20 | 42 | 19 | 1.92 | 10 | 13 | 2 |
| G27 | BUT06xBUT09 | 0.34 | 21 | 23 | 5 | 2.43 | 31 | 68 | 39 | 1.88 | 9 | 17 | 3 |
| G28 | BUT10xBUT11 | 0.33 | 20 | 31 | 11 | 1.33 | 13 | 33 | 12 | 2.70 | 16 | 25 | 8 |
| G29 | BUT12 | 0.07 | 3 | 21 | 3 | 3.06 | 36 | 74 | 40 | 4.66 | 31 | 69 | 36 |
| G30 | HYB01# | 0.44 | 27 | 49 | 31 | 1.21 | 11 | 30 | 11 | 4.65 | 30 | 52 | 32 |
| G31 | BUT13 | 0.48 | 31 | 73 | 41 | 2.85 | 34 | 44 | 23 | 1.64 | 8 | 45 | 25 |
| G32 | BUT14 | 0.20 | 11 | 24 | 6 | 1.58 | 21 | 57 | 33 | 5.10 | 33 | 63 | 35 |
| G33 | BUT15 | 0.16 | 9 | 35 | 15 | 1.37 | 15 | 38 | 18 | 0.66 | 2 | 35 | 15 |
| G34 | OPV01 | 0.35 | 22 | 58 | 33 | 2.25 | 30 | 60 | 35 | 2.12 | 11 | 50 | 30 |
| G35 | HYB02# | 0.54 | 36 | 44 | 22 | 2.19 | 28 | 52 | 30 | 7.47 | 40 | 46 | 26 |
| G36 | HYB03 | 0.11 | 6 | 35 | 15 | 1.98 | 25 | 36 | 15 | 4.34 | 26 | 49 | 27 |
| G37 | HYB04# | 0.48 | 33 | 63 | 36 | 3.43 | 37 | 53 | 32 | 6.05 | 36 | 61 | 34 |
| G38 | HYB05# | 0.21 | 14 | 46 | 27 | 2.56 | 33 | 37 | 17 | 6.35 | 37 | 49 | 27 |
| G39 | HYB06# | 0.09 | 4 | 25 | 7 | 2.25 | 29 | 42 | 19 | 3.73 | 21 | 35 | 15 |
| G40 | OPV02 | 0.33 | 19 | 44 | 22 | 3.03 | 35 | 67 | 38 | 1.59 | 7 | 41 | 23 |
| G41 | HYB07# | 0.39 | 25 | 34 | 14 | 0.70 | 6 | 34 | 14 | 4.62 | 29 | 40 | 21 |
| G42 | HYB08# | 0.45 | 28 | 68 | 39 | 5.14 | 42 | 43 | 22 | 2.19 | 12 | 31 | 13 |

# Commercially available in South Africa; RASV: Rank of the ASV, RYSI: Rank of the YSI; Top 10 and bottom 10 ranked genotypes are highlited in green and orange respectively

**Table 4.3** AMMI stability value (ASV) and yield stability index (YSI) for dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), and total soluble solids (TSS) for 42 genotypes

| | | DMC | | | | Fruit a* | | | | TSS | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Entry | Genotype | ASV | RASV | YSI | RYSI | ASV | RASV | YSI | RYSI | ASV | RASV | YSI | RYSI |
| G1 | BUT01 | 0.51 | 22 | 38 | 14 | 0.37 | 8 | 44 | 24 | 0.61 | 28 | 47 | 29 |
| G2 | BUT02 | 0.90 | 35 | 54 | 32 | 0.35 | 5 | 15 | 3 | 0.97 | 36 | 60 | 39 |
| G3 | BUT03 | 0.52 | 23 | 30 | 8 | 0.75 | 23 | 42 | 21 | 0.37 | 13 | 22 | 3 |
| G4 | BUT04 | 0.07 | 2 | 36 | 12 | 0.59 | 18 | 38 | 15 | 0.45 | 17 | 43 | 20 |
| G5 | BUT05 | 0.24 | 8 | 49 | 29 | 0.36 | 7 | 47 | 27 | 0.19 | 2 | 39 | 14 |
| G6 | BUT06 | 1.63 | 41 | 43 | 20 | 0.94 | 29 | 33 | 12 | 1.14 | 38 | 40 | 15 |
| G7 | BUT07 | 0.62 | 27 | 28 | 6 | 1.52 | 37 | 40 | 18 | 0.81 | 35 | 36 | 10 |
| G8 | BUT08 | 1.10 | 36 | 56 | 35 | 0.98 | 31 | 33 | 12 | 0.79 | 31 | 46 | 28 |
| G9 | BUT01xBUT05 | 0.38 | 15 | 43 | 20 | 1.03 | 32 | 66 | 38 | 0.30 | 8 | 40 | 15 |
| G10 | BUT02xBUT05 | 0.47 | 21 | 56 | 35 | 1.03 | 33 | 58 | 33 | 0.48 | 19 | 58 | 38 |
| G11 | BUT03xBUT05 | 0.37 | 14 | 40 | 17 | 0.98 | 30 | 59 | 34 | 0.47 | 18 | 49 | 30 |
| G12 | BUT04xBUT05 | 0.28 | 9 | 38 | 14 | 1.05 | 34 | 65 | 37 | 0.43 | 16 | 45 | 25 |
| G13 | BUT01xBUT06 | 0.63 | 28 | 42 | 19 | 0.24 | 1 | 22 | 5 | 0.21 | 3 | 19 | 2 |
| G14 | BUT02xBUT06 | 0.36 | 13 | 28 | 6 | 0.55 | 16 | 29 | 10 | 0.59 | 27 | 45 | 25 |
| G15 | BUT03xBUT06 | 1.17 | 37 | 45 | 25 | 1.16 | 35 | 53 | 32 | 0.49 | 21 | 31 | 7 |
| G16 | BUT04xBUT06 | 0.03 | 1 | 19 | 1 | 0.57 | 17 | 26 | 8 | 0.48 | 20 | 34 | 9 |
| G17 | BUT01xBUT07 | 0.54 | 25 | 30 | 8 | 0.76 | 24 | 39 | 16 | 0.55 | 26 | 33 | 8 |
| G18 | BUT02xBUT07 | 0.78 | 32 | 43 | 20 | 0.43 | 12 | 23 | 7 | 0.80 | 33 | 44 | 22 |
| G19 | BUT03xBUT07 | 0.83 | 33 | 37 | 13 | 0.89 | 27 | 41 | 19 | 0.81 | 34 | 38 | 12 |
| G20 | BUT04xBUT07 | 0.38 | 16 | 26 | 3 | 0.29 | 4 | 10 | 1 | 0.30 | 7 | 15 | 1 |
| G21 | BUT01xBUT08 | 0.31 | 10 | 39 | 16 | 0.50 | 14 | 47 | 27 | 0.53 | 25 | 55 | 37 |
| G22 | BUT02xBUT08 | 0.20 | 4 | 43 | 20 | 0.89 | 28 | 51 | 31 | 0.38 | 14 | 54 | 35 |
| G23 | BUT03xBUT08 | 0.40 | 18 | 45 | 25 | 0.60 | 19 | 46 | 25 | 0.22 | 4 | 38 | 12 |
| G24 | BUT04xBUT08 | 0.22 | 6 | 30 | 8 | 0.36 | 6 | 22 | 5 | 0.38 | 15 | 40 | 15 |
| G25 | BUT09 | 0.77 | 31 | 56 | 35 | 2.69 | 42 | 84 | 42 | 0.69 | 29 | 52 | 32 |
| G26 | BUT03xBUT09 | 0.60 | 26 | 61 | 41 | 0.86 | 26 | 67 | 39 | 0.31 | 11 | 44 | 22 |
| G27 | BUT06xBUT09 | 0.88 | 34 | 55 | 34 | 1.64 | 40 | 79 | 41 | 0.80 | 32 | 49 | 30 |
| G28 | BUT10xBUT11 | 0.31 | 11 | 43 | 20 | 0.67 | 22 | 59 | 34 | 0.31 | 10 | 37 | 11 |
| G29 | BUT12 | 0.22 | 5 | 27 | 4 | 0.40 | 9 | 41 | 19 | 0.27 | 6 | 28 | 4 |
| G30 | HYB01# | 0.16 | 3 | 20 | 2 | 0.27 | 3 | 20 | 4 | 0.31 | 9 | 30 | 6 |
| G31 | BUT13 | 0.54 | 24 | 27 | 4 | 0.84 | 25 | 26 | 8 | 0.50 | 22 | 28 | 4 |
| G32 | BUT14 | 0.76 | 30 | 53 | 31 | 0.50 | 15 | 50 | 30 | 1.24 | 40 | 60 | 39 |
| G33 | BUT15 | 0.39 | 17 | 30 | 8 | 1.58 | 38 | 43 | 23 | 0.70 | 30 | 42 | 19 |
| G34 | OPV01 | 1.31 | 39 | 48 | 27 | 1.85 | 41 | 71 | 40 | 1.15 | 39 | 44 | 22 |
| G35 | HYB02# | 0.33 | 12 | 54 | 32 | 0.42 | 10 | 48 | 29 | 0.17 | 1 | 43 | 20 |
| G36 | HYB03 | 0.23 | 7 | 40 | 17 | 0.65 | 21 | 42 | 21 | 0.26 | 5 | 40 | 15 |
| G37 | HYB04# | 0.44 | 20 | 60 | 40 | 0.63 | 20 | 32 | 11 | 0.32 | 12 | 53 | 34 |
| G38 | HYB05# | 0.41 | 19 | 57 | 38 | 0.42 | 11 | 39 | 16 | 0.50 | 23 | 61 | 41 |
| G39 | HYB06# | 1.22 | 38 | 75 | 42 | 1.29 | 36 | 62 | 36 | 1.08 | 37 | 73 | 42 |
| G40 | OPV02 | 1.69 | 42 | 48 | 27 | 1.61 | 39 | 46 | 25 | 1.68 | 42 | 45 | 25 |
| G41 | HYB07# | 0.70 | 29 | 58 | 39 | 0.45 | 13 | 37 | 14 | 0.51 | 24 | 52 | 32 |
| G42 | HYB08# | 1.42 | 40 | 52 | 30 | 0.27 | 2 | 10 | 1 | 1.57 | 41 | 54 | 35 |

# Commercially available in South Africa; RASV: Rank of the ASV, RYSI: Rank of the YSI; Top 10 and bottom 10 ranked genotypes are highlited in green and orange respectively

ASV was used to quantify stability of genotypes for all traits. The genotypes were grouped according to high, medium and low stability using the first and second standard deviations, and the remainder of the genotypes, respectively, as categories. These ASV categories, in combination with mean performance of each genotype are summarised in a bullet graph (Figure 4.2).

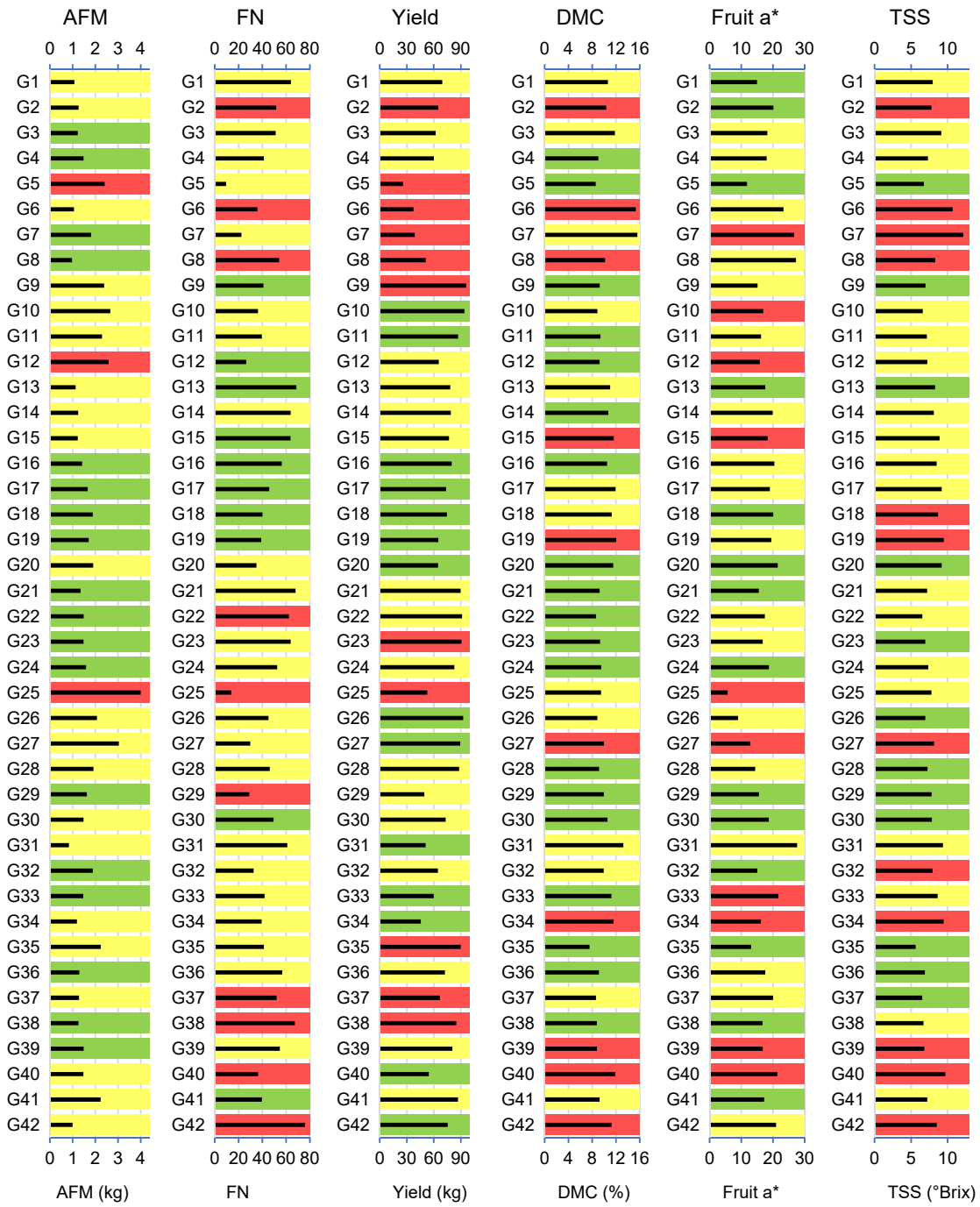


**Figure 4.2** Bullet graph summary of stability and mean performance of 42 butternut genotypes evaluated in environments for average fruit mass (AFM), fruit number (FN), yield, total soluble solids (TSS), dry matter content (DMC) and green-red colour contribution in fruit mesocarp (Fruit a*). The horizontal bars represent genotypes (G1 to G42) where green, yellow and red background fill colour represent high, medium and low stability, respectively. The length of the horizontal black line in each horizontal bar is an indication of the trait mean. A key to the labels of genotypes is available in Table 4.2.

### 4.4.3 Scatter plot view of the GGE biplot

The GGE scatter plots revealed 96%, 90%, 90%, 88%, 89% and 91% of the G x E interaction variation for AFM, FN, yield, TSS, DMC and Fruit a* respectively (Figure 4.3). FN (Figure 4.3B), yield (Figure 4.3C), DMC (Figure 4.3E) and Fruit a* (Figure 4.3F) revealed different mega-environments, confirming the existence of crossover G x E interactions. Both AFM (Figure 4.3A) and TSS (Figure 4.3D) featured only one mega-environment, where G25 produced the largest fruit and G7 the highest TSS respectively across all the environments. Two mega-environments each were identified for FN, yield and DMC. For DMC G7 was the best performer in the E1, E2 and E3 mega-environment and G6 best in the E4, E5 and E6 mega-environment. The first mega-environment for both FN and yield consisted of E1, E3, E4 and E5, with the best performers being G42 and G9 respectively. Genotypes G13 and G21 were the best performers for FN and yield respectively in a second mega-environment, consisting of E2 and E6. Three mega-environments were identified for Fruit a*. Genotype G8 was the best performer in the E1 and E3 mega-environment and G7 and G31 were the best in the E6 mega-environment. Since the E2, E4 and E5 mega-environment does not have a vertex genotype, G8, G31 and G7 could be expected to be superior performers under these conditions.

### 4.4.4 Ranking and stability view of the GGE biplot

Plant breeders are interested mostly in stability associated with a high trait mean. These genotypes are located on the high-performance end of the GGE biplot, nearest to the AEA. In terms of AFM, G25 had the largest fruit, followed by G27 and G10. G5, followed by G12 were the least stable (Figure 4.4A). In the case of FN (Figure 4.4B), G42 had the highest number but was relatively unstable. In contrast, G5 produced the smallest amount of fruit but was stable. Genotypes G21 and G13 were both highly stable with high numbers of fruit. For yield, G10 and G9 performed the best, with G10 being the most stable of the two (Figure 4.4C). Genotype G2 was the least stable for yield and G5 showed both low yield and low stability. With regard to internal quality, G7 and G6 were the best performers for TSS with both relatively stable performance (Figure 4.4D). In the case of DMC, G7 and G6 featured again as the highest performers although G7 was the most stable of the two (Figure 4.4E). When looking at both TSS and DMC, G40 was one of the least stable genotypes and G35 was the worst performer, although relatively stable. Genotypes G31, G8 and G7 had the highest ranking for Fruit a*, while G25 had both poor fruit colour and low stability (Figure 4.4F).

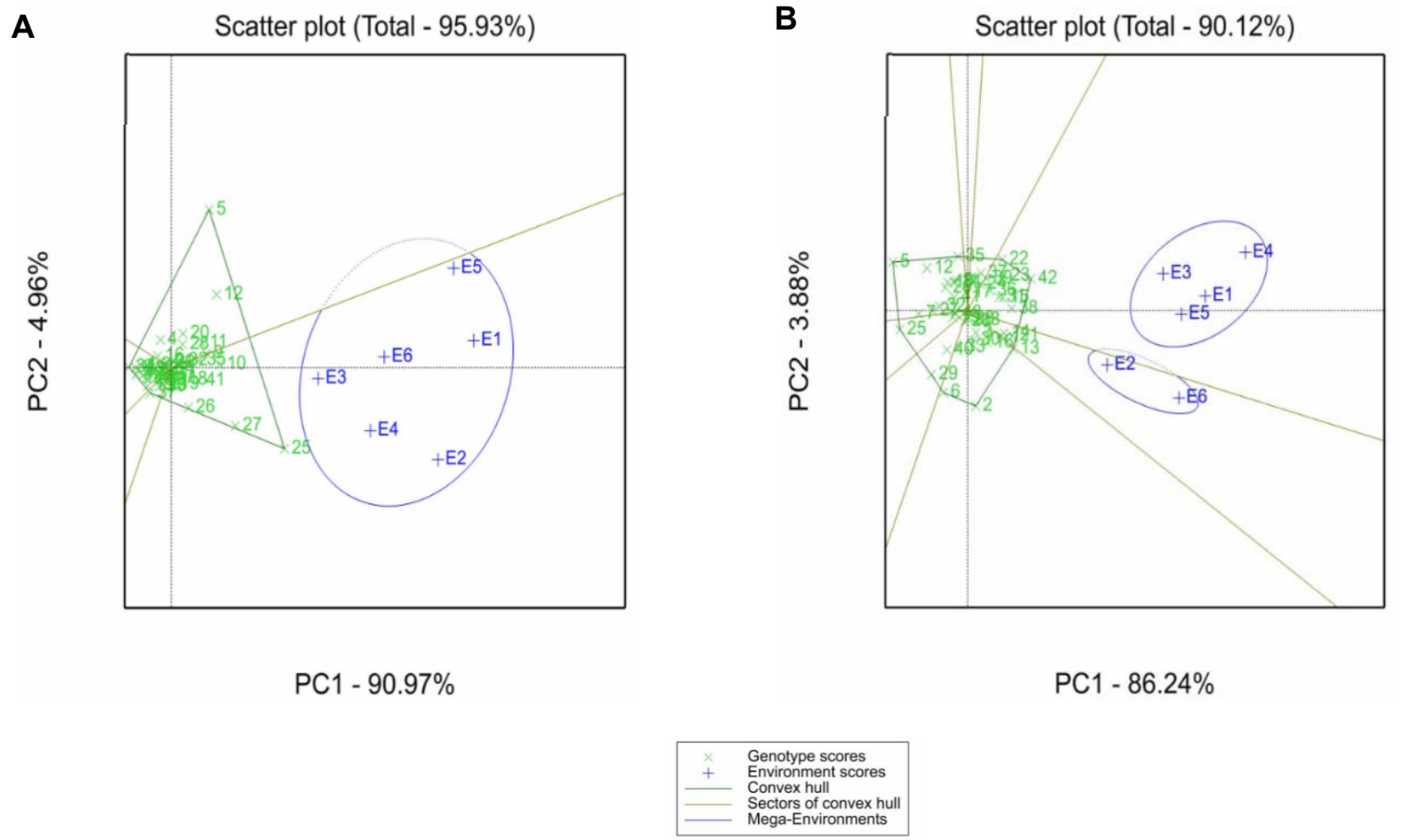


**Figure 4.3** GGE biplots (scatter plot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. A: average fruit mass (AFM), B: fruit number (FN), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020

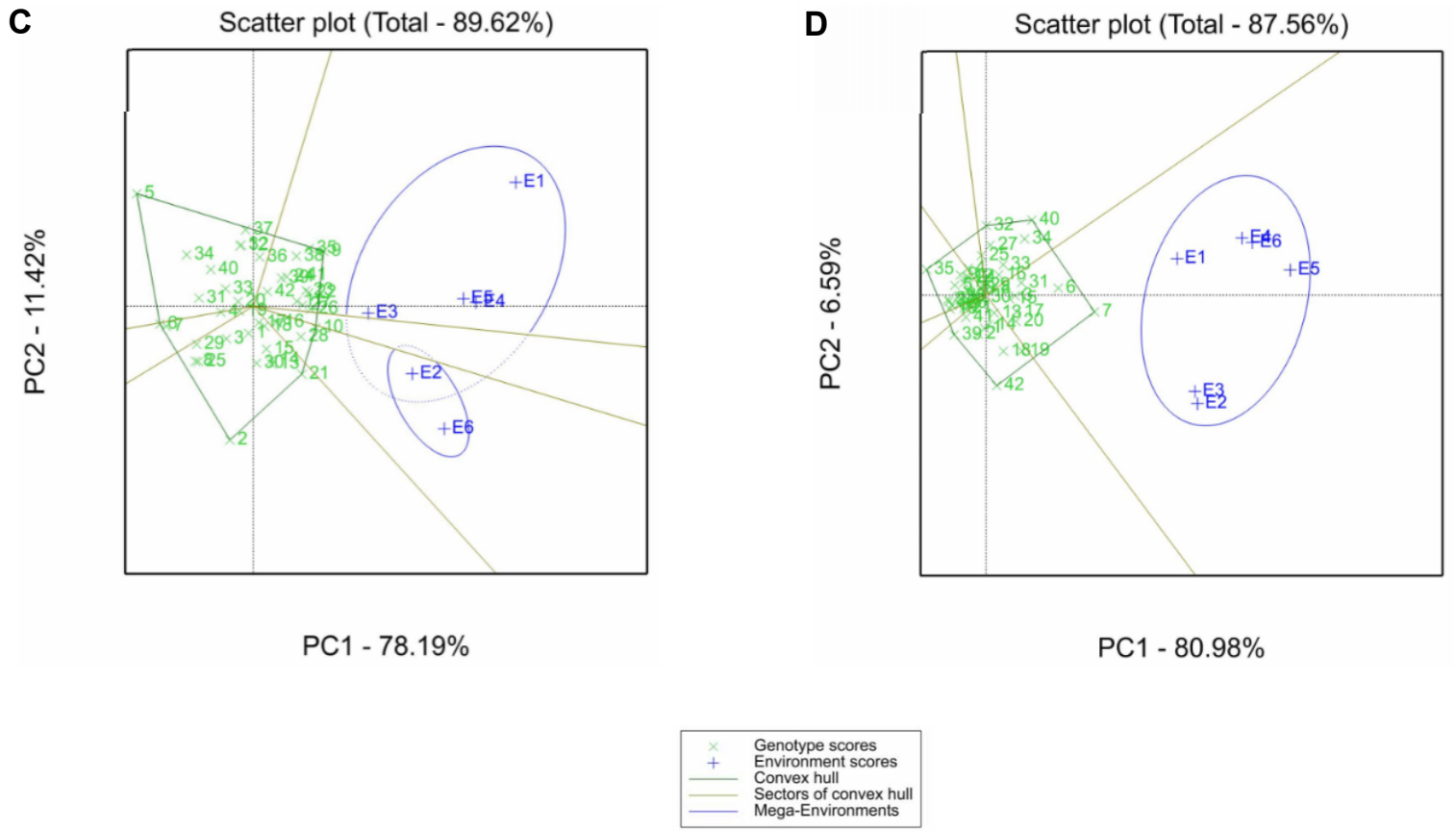


**Figure 4.3** GGE biplots (scatter plot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. C: yield, D: total soluble solids (TSS), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020

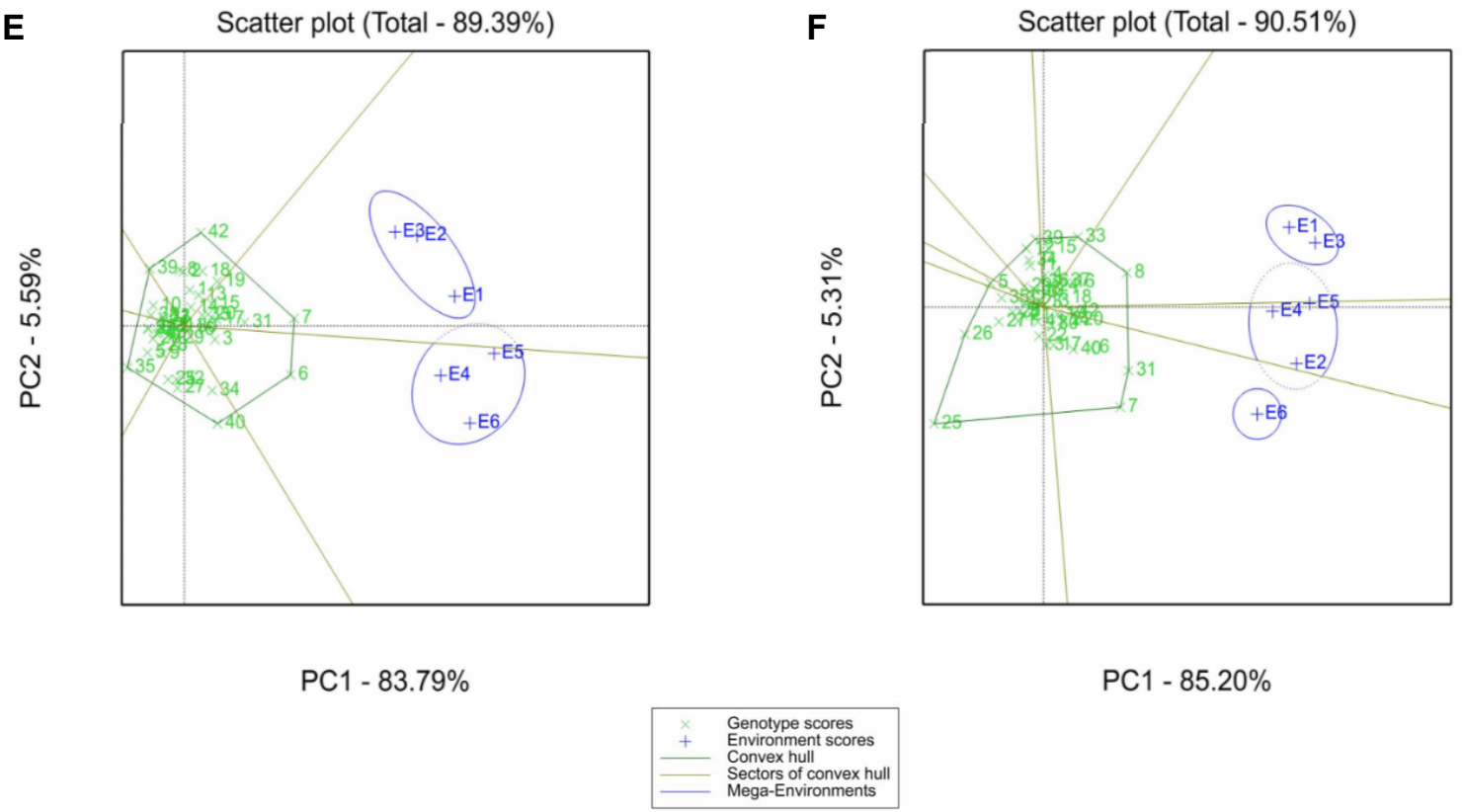


**Figure 4.3** GGE biplots (scatter plot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. E: dry matter content (DMC), F: green-red colour contribution in fruit mesocarp (Fruit a*), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020

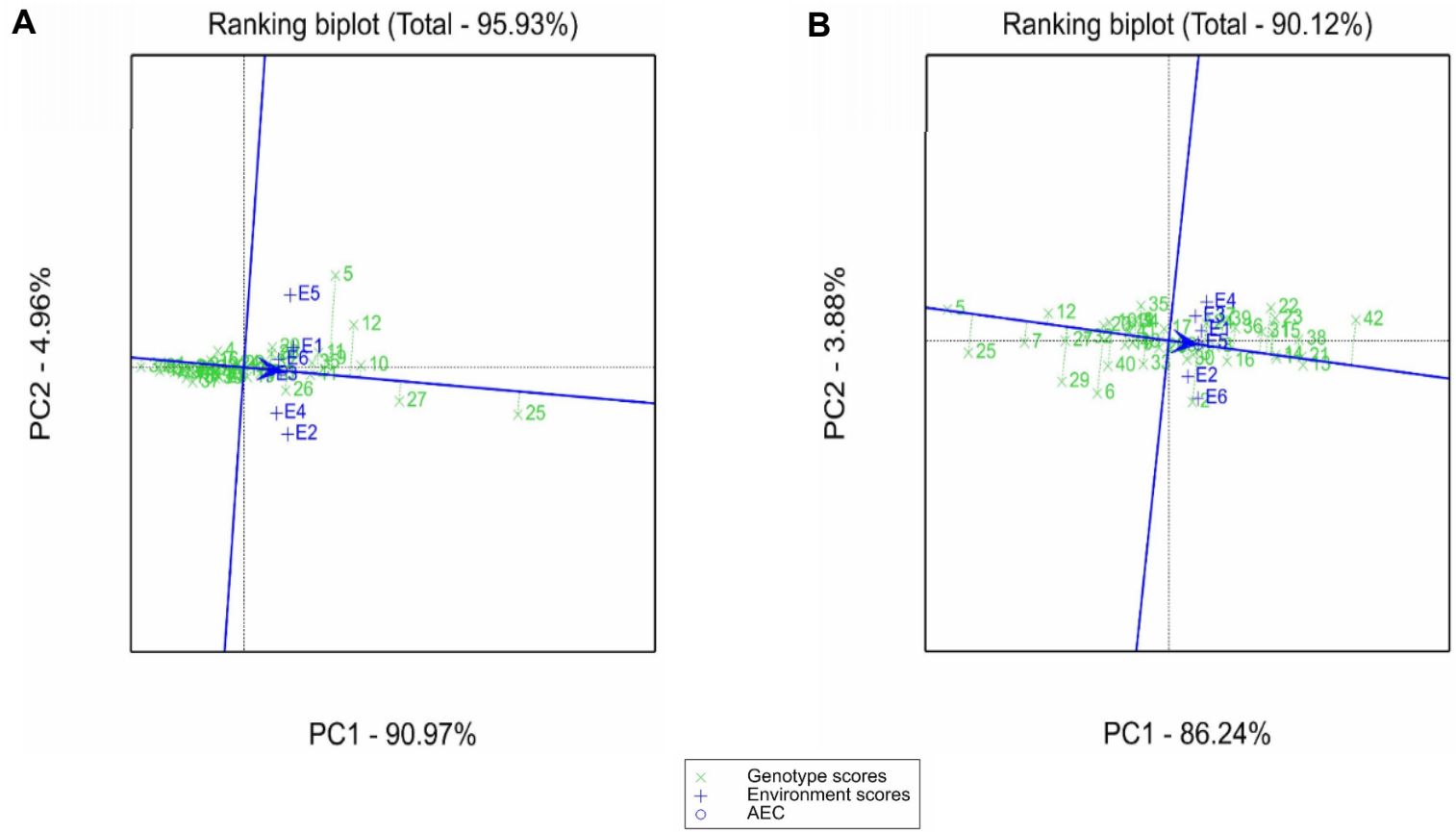


**Figure 4.4** GGE biplots (ranking biplot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. A: average fruit mass (AFM), B: fruit number (FN), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020, AEC: average environment coordinate

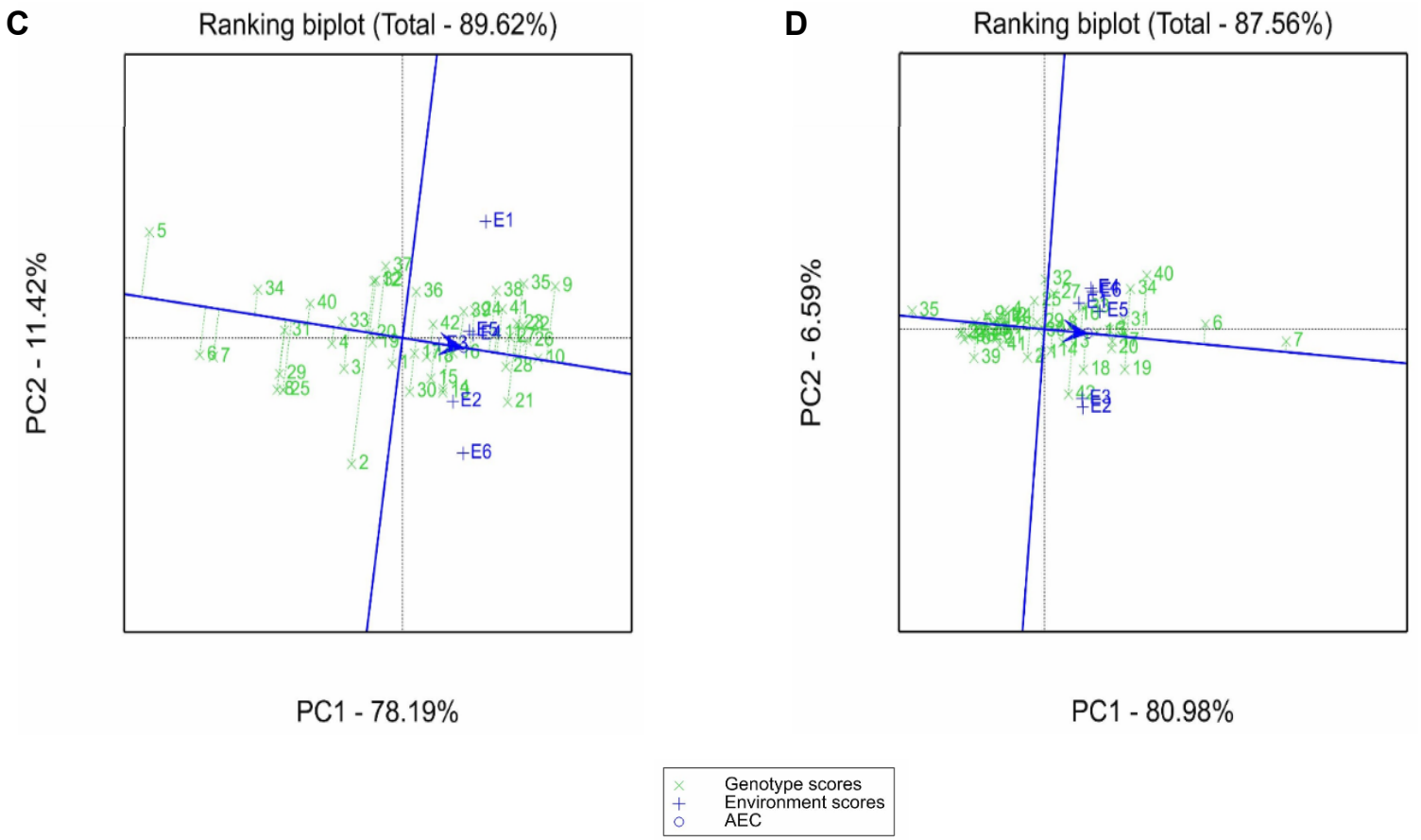


**Figure 4.4** GGE biplots (ranking biplot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. C: yield, D: total soluble solids (TSS), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020, AEC: average environment coordinate

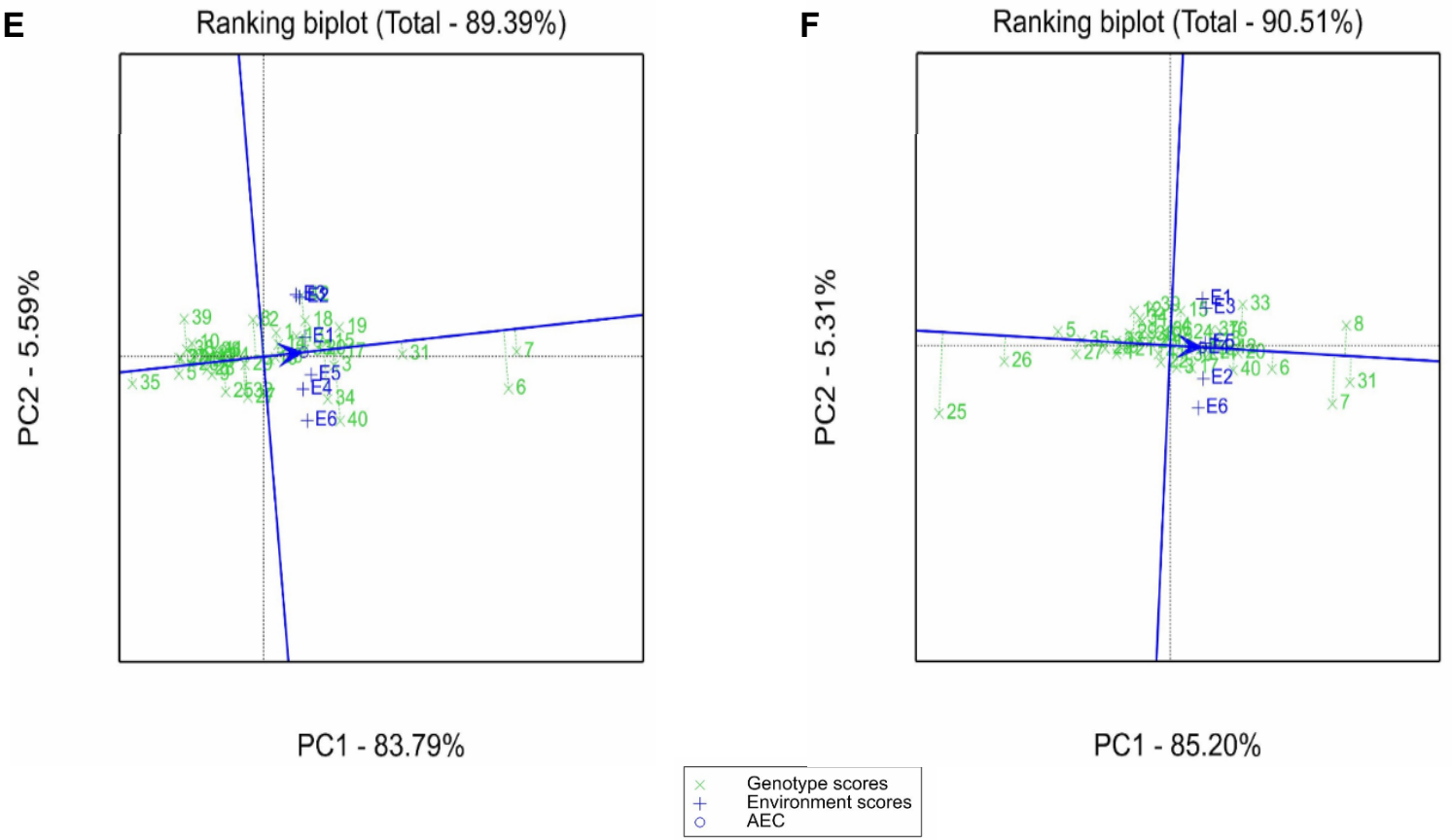


**Figure 4.4** GGE biplots (ranking biplot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. E: dry matter content (DMC), F: green-red colour contribution in fruit mesocarp (Fruit a*), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020, AEC: average environment coordinate

### 4.4.5 Genotype comparison with ideal genotype view of the GGE biplot

In this study the most desirable genotypes for AFM, FN and Fruit a* were G25, G42 and G8, respectively (Figure 4.5A, B, F). For yield, a greater number of more genotype markers were concentrated closer to the ideal genotype location with G10 and G26 both positioned on the first inner circle (Figure 4.5C). Genotype G7 emerged as the most desirable genotype for both TSS and DMC (Figure 4.5D, E).

## 4.5 Discussion

Wide variation across a range of characteristics in *C. moschata* was once again demonstrated in this study. The combined ANOVA across seasons and locations, using the AMMI model, resulted in non-significant G x E interaction effects for FN and yield (results not shown). This was unexpected since the ANOVA across seasons and locations in Chapter 3 presented significant genotype x location, genotype x season and genotype x location x season interactions. A possible explanation was that some of the variation was removed when values across the two seasons were averaged when used for the AMMI analysis. Subsequent analyses used six environments where every location within a specific season represented a single environment. These analyses confirmed that genotypes responded inconsistently across environments, due to significant G x E interaction.

Although the environment played a significant role in phenotypic variation, most of the variation was attributed to genetic variation for all the traits evaluated with the exception of yield. This implied that significant progress could be made by selection of superior individuals, but the significant environmental effects and G x E interactions still cautioned that multi-location trials are needed to confirm stability and adaptability of genotypes across environments for all the characteristics of interest. The high genotype effect, with low environment and G x E interaction effects for DMC, Fruit a* and TSS may necessitate evaluation across fewer environments when compared to yield and its components, in order to distinguish desirable genotypes. For AFM, FN and more specifically yield, much larger differences can be expected across different environments. Similar results were obtained in butternut (Valdes-Restrepo *et al.,* 2013) cassava (Peprah *et al.,* 2016) and sweet potato (Tumwegamire *et al.*, 2016), where DMC was demonstrated to be more stable than yield across multiple environments.

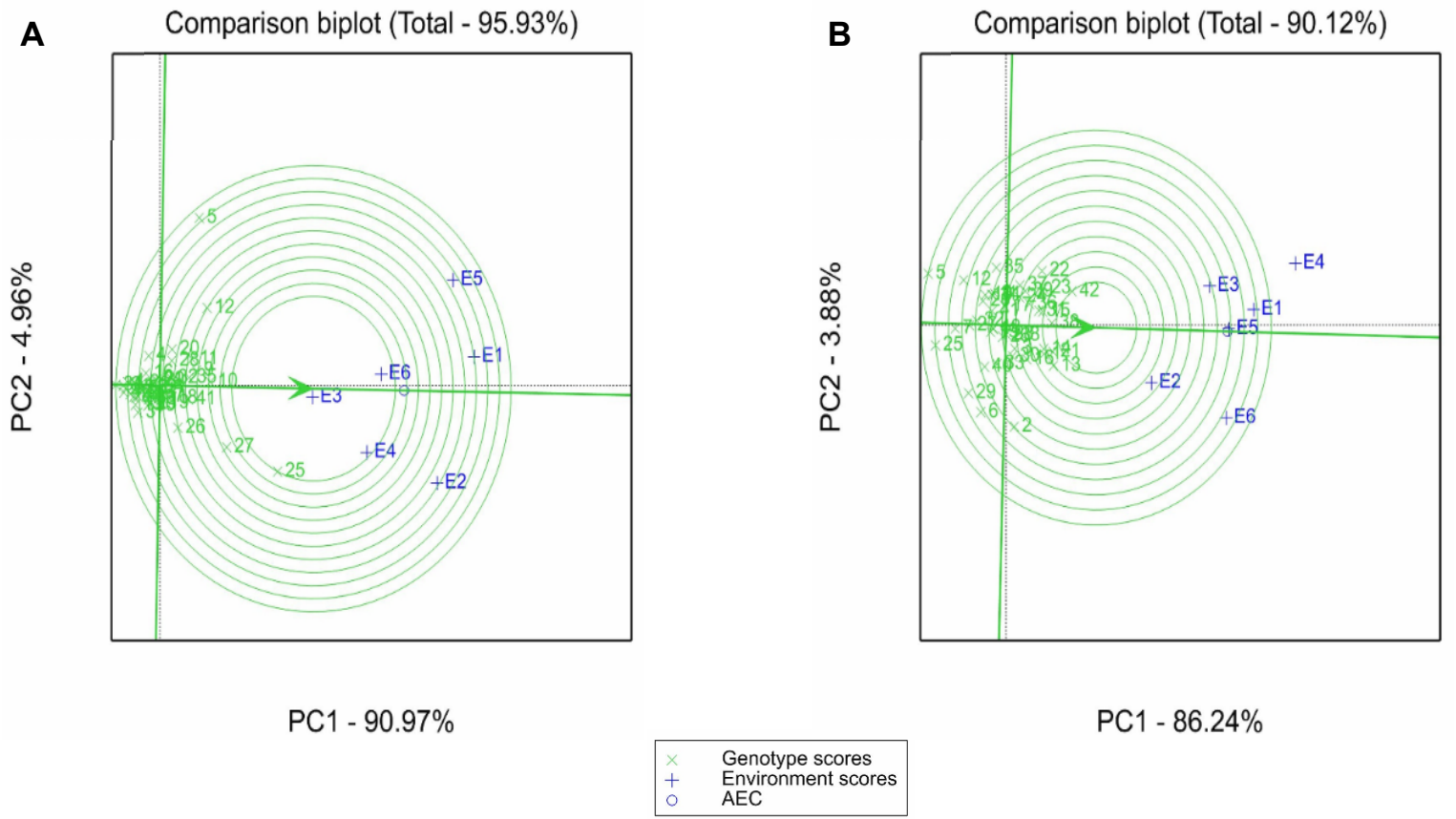


**Figure 4.5** GGE biplots (comparison biplot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. A: average fruit mass (AFM), B: fruit number (FN), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020, AEC: average environment coordinate

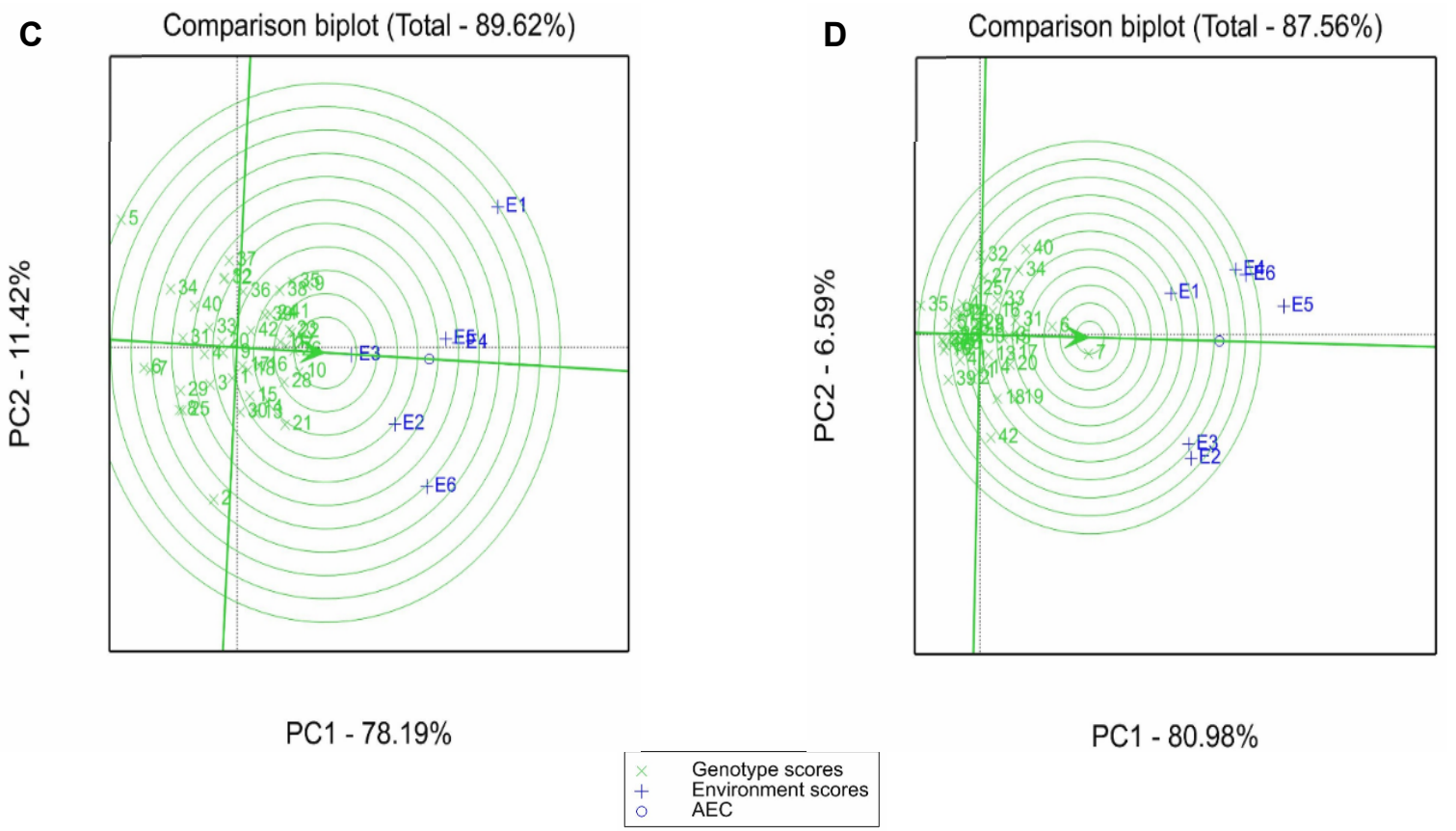


**Figure 4.5** GGE biplots (comparison biplot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. C: yield, D: total soluble solids (TSS), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020, AEC: average environment coordinate

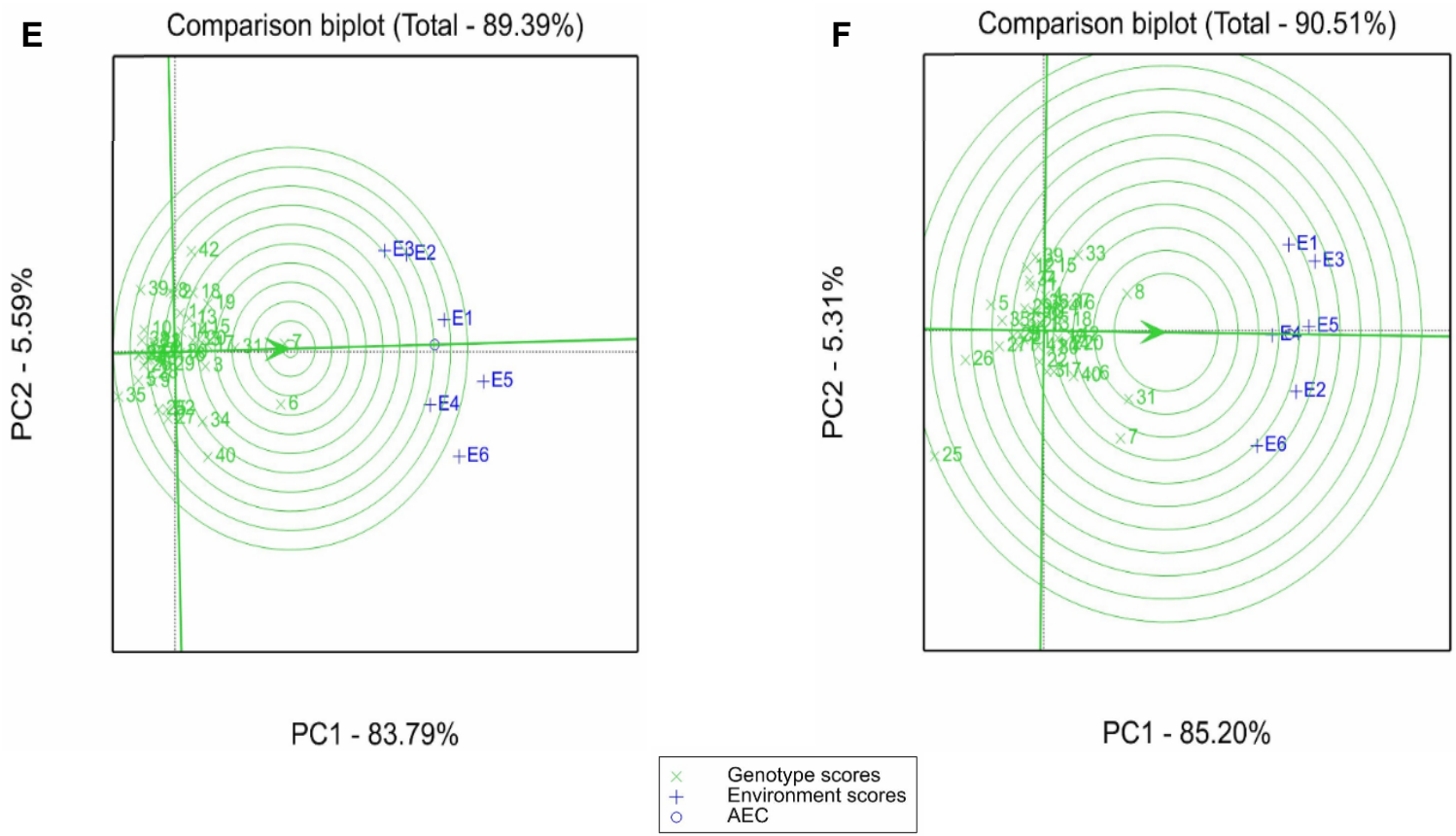


**Figure 4.5** GGE biplots (comparison biplot view) showing the main and first interaction principal components (IPCA1) of selected morpho-agronomic and internal quality characteristics. E: dry matter content (DMC), F: green-red colour contribution in fruit mesocarp (Fruit a*), G1 to G42: genotypes, E1: Jacobsdal 2018/2019, E2: Jacobsdal 2019/2020, E3: Kaalfontein 2018/2019, E4: Kaalfontein 2019/2020, E5: Oudtshoorn 2018/2019, E5: Oudtshoorn 2019/2020, AEC: average environment coordinate

A mega-environment is defined as a group of locations that consistently share the best set of genotypes across environments (Yan *et al.,* 2007). In this study, environments representing different locations from different years were associated with different mega-environments. It can therefore be assumed that mega-environment patterns across years will be different. The G x E interactions can be exploited by selecting for specific mega-environments; however, if the crossover G x E interaction pattern is not consistent over years this must rather be avoided, and selection for best performing and most stable genotypes across all environments (across years) is preferable (Yan and Tinker, 2006).

There are two approaches in breeding with which to reduce G x E interactions in material. The first is to sub-divide heterogeneous environments into more homogeneous sub-regions so that material adapted to specific environments can be developed. This strategy is not suitable in butternut since the seed market size is not big enough to support a large number of different varieties. An alternative approach would be to reduce G x E interactions by developing stable material that is adapted to a wide range of environments (Farshadfar *et al.,* 2011). A similar approach is followed for pickling cucumber (Dia *et al.,* 2018) and pepper (Barchenger *et al.,* 2018), where breeders aim to develop varieties adapted to diverse rather than single regions.

When comparing AMMI and GGE biplot analyses, the GGE biplot clearly captured more variation for all butternut traits evaluated in this study, presumably since the genotype main effect had been removed in the AMMI analysis. This was consistent with studies in watermelon (Dia *et al.* 2016) and cassava (Peprah *et al.,* 2016; 2020). Even so, both the AMMI and GGE biplot analyses successfully captured a large part of the genotype and G x E interactions for all characteristics evaluated, making it possible to identify desirable genotypes for each of the characteristics evaluated.

High-performing genotypes accompanied by high stability are usually widely adapted across most environments and genotypes with medium stability are adapted to more specific environments. Since it is rare to find a single genotype that is desirable for all characteristics, plant breeders are forced to select not only the most desirable genotype but rather a range of genotypes with acceptable yield and stability across all characteristics. This allows identification of specific genotypes with acceptable performance and stability for all economically important traits. In that regard, this study was no different, with no single genotype widely adapted for all six characteristics analysed emerging, but there were various genotypes that exhibited combined medium- or high-stability for all traits. It should also be noted that in most instances, more than 25% of the G x E interaction variation was not

accounted for by the IPCAs and, therefore, these values should be used as an indication only, with possible deviations from predicted scenarios to be expected.

In addition, market segments are often determined by fruit sizes; thus, poor AFM performance is not always a disqualifying characteristic. Fruit size is used to allocate a genotype to a specific market segment only, however, stability of AFM is crucial to produce a consistent product with regards to fruit size. Consistent high performance of other yield components is also important for commercial growers, while DMC, Fruit a* and TSS are more important for processors and the domestic consumer. Any genotype with either low performance or low stability for any of those characteristics should preferably be avoided, and should only be used as sources of specific advantageous traits such as disease resistance and specific quality characteristics. Stability of G2, G6 and G8 was low across FN, yield, DMC and TSS. Genotype G25 was unstable for all yield component characteristics as well as Fruit a*. Genotype G40 consistently performed average for yield, but it was unstable for FN, DMC, Fruit a* and TSS. All genotypes exhibiting low stability for four of the six characteristics happened to be either parental lines or open-pollinated varieties adapted to specific environmental conditions. This has also been observed in a watermelon study where hybrids had advantages over inbred lines for yield components in both performance and responsiveness to favourable environments (Dia *et al.,* 2016).

In Chapter 3, a number of genotypes were recommended for specific market segments based on performance of various characteristics. Genotype G9 was recommended for the processing industry due to its extremely high yields, but the stability analysis showed the genotype is unstable. Yield of G11 was significantly lower than that of G9, but not significantly lower than the commercial standards viz., G35 and G41. Stability of G11 was medium across all characteristics evaluated, with higher yield stability than G35. The DMC, TSS, Fruit a* and penetrometer reading (PEN) of G11 were not significantly different from G41 for DMC, TSS, Fruit a* and PEN, but was significantly better than G35 for the same internal traits.

For the fresh market segment, four hybrids were identified as better performers than the commercial standards. Of those, G18 was not stable for TSS, making it less desirable. Stability for yield of G23 was medium only, and although this would actually be acceptable in most of the environments, there are more stable genotypes available. Yield component characteristics of G16 and G17 were both highly stable. In terms of quality characteristics, the two hybrids are similar, except in the case of DMC where G16 was more stable.

For the small-fruited market segment, G15 was unstable for DMC and flesh colour. With the exception of DMC that demonstrated high stability, G14 showed medium stability for all the traits evaluated. High stability for flesh colour, FN and TSS made G13 more desirable, since it was adapted to a wider range of environments.

Of the commercially available material in particular, it is clear that some of the most popular commercial genotypes were also the least stable. For example, G35, G37 and G39 all had very low yield stability. The only commercial hybrid with high yield stability was G42, where the only trait for which low stability was not measured was AFM, indicating the importance of consistent fruit size for specific market segments.

### 4.6 Conclusions

Several butternut genotypes in this study exhibited significant G x E interactions with regard to yield and a number of quality characteristics. These interactions were successfully visualised using AMMI and GGE biplots, where genotypes revealed high yields and desirable quality performance, as well as high stability for the various characteristics. The most desirable genotypes for the various butternut market segments included G11 for the processing segment, G16 and G17 for the fresh market segment and G13 for the small-fruited market segment. Since high performance and stability were not necessarily combined within the same genotypes, there remain opportunities for improvement in developing suitable genotypes with low G x E interactions for economically important traits, resulting in high-performing, stable phenotypes across a wide range of environmental conditions.

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# CHAPTER 5

# GENETIC COMPONENTS, CORRELATIONS AND PATH COEFFICIENT ANALYSIS OF MORPHO-AGRONOMIC AND INTERNAL FRUIT QUALITY CHARACTERISTICS IN BUTTERNUT


## 5.1 Abstract

Untill now, the genetic variability within Starke Ayres butternut germplasm and its potential applications to breeding strategies have not been thoroughly described. The aims of this study were to estimate the genetic parameters in Starke Ayres butternut germplasm in order to establish selection feasibility as well as to evaluate potential correlations between 15 morpho-agronomic and internal fruit quality characteristics. Twenty-seven *Cucurbita moschata* $F_1$ hybrids across three locations and two seasons were evaluated. Warm and dry environments were represented by Jacobsdal and Oudshoorn, and a more humid, temperate environment by Kaalfontein. A high level of broad-sense heritability, coupled with moderate to high genetic gain as a percentage of the population mean, was observed for average fruit mass (AFM), fruit number (FN), total soluble solids (TSS), dry matter content (DMC), fruit mesocarp colour and mesocarp firmness. These characteristics appeared to be under additive genetic control and simple selection for these traits in early generations are likely to be effective. In contrast, results for green-red colour contribution in the leaf canopy, dry matter yield, uniformity and fruit yield suggested that simple selection alone would not be effective. Leaf chlorophyll content (CHL), yellow-blue colour contribution in the leaf canopy, leaf width (LW), petiole length and internal breakdown of fruit mesocarp displayed a moderately high level of broad-sense heritability, with lower genetic gain as a percentage of the mean. This indicated the presence of additive genetic control, although the environment played a more significant role in the expression of these phenotypes. Genotypic and phenotypic correlations indicated a strong negative association between AFM and FN, and a strong positive correlation between DMC and TSS. CHL had moderate correlations with AFM and FN, while weak correlations were found between yield and TSS, as well as DMC and the green-red colour contribution to fruit mesocarp colour. A path coefficient analysis indicated recurring direct effects on yield by FN, AFM, internal breakdown of fruit mesocarp and LW. A focus on yield, FN and AFM, in combination with DMC and TSS, is suggested as the most suitable strategy for a breeding programme aimed at improving fruit yield accompanied by high levels of internal fruit quality.



***Keywords:*** Broad-sense heritability, *Cucurbita moschata*, correlation, genetic advance, genotypic coefficient of variation, variability

## 5.2 Introduction

Worldwide, the genus *Cucurbita* is grown as an economically important crop comprising of probably five domesticated species. It is estimated that the total area under cultivation and the total world production of *C. moschata*, *C. pepo* and *C. maxima* collectively in 2018 was approximately 2 million hectares and 27 million tons, respectively (FAO, 2018). Diversity in *C. moschata* continues to be conserved in the form of numerous landraces across many countries (Lee *et al*., 2021). Butternut typically expresses a high level of genetic variability for fruit shape and colour, as well as numerous internal fruit quality characteristics (Hazra *et al*., 2007; Du *et al*., 2011). Due to the nutritional value of the mesocarp of *C. moschata*, it plays an important role in the human diet (Gomes *et al.*, 2020). Any improvement to the nutritional component of vegetables, including cucurbits, through breeding is a laudable strategy that seeks to reduce malnourishment, while promoting food security at the same time (Shafiin *et al*., 2020).

Fruit shapes vary greatly across the different regions of the globe, with nations having very specific preferences for particular fruit shapes and fruit quality (Noseworthy and Loy, 2008). The typical butternut shape we are familiar with is actually a recent occurrence (Mutschler and Pearson, 1987) and differs quite significantly from the more traditional globular crookneck and flattened landrace pumpkin shapes (Hazra *et al*., 2007; Gomes *et al.*, 2020). All of these morphological characteristics, together with genetic markers, could group the types into various clusters (Du *et al*., 2011; Nagar *et al*., 2017). The butternut type occupies only a small part of the diversity within *C. moschata* and genetic parameters specific to that type will need to be established prior to any exploitation of the variation for any morpho-agronomic or internal fruit quality characteristics for crop improvement.

Successful breeding programmes depend not only on the degree of genetic variation that exists within a crop but also on the heritability i.e., the amount of variation that can be transferred from the parents to the progeny. Genetic advance is also an important indication of the degree of genetic gain that can be expected for a specific characteristic through a single cycle of selection (Rosmaina *et al*., 2016). Determining these two genetic parameters would, therefore, be an important preliminary step when designing an effective approach in any breeding programme (Al-Tabbal and Al-Fraihat, 2012).

Similarly, correlation coefficients are important in plant breeding since they indicate the genetic and non-genetic association between two or more traits. In addition, the associations quantify possible gains due to indirect selection within correlated traits. Path coefficient analysis is used

in order to partition correlations for a specific characteristic into both direct and indirect effects (Silva *et al*., 2016).

Numerous *C. moschata* studies have explored genetic parameters and correlations with yield components (Kumar *et al*., 2011; Akter *et al*., 2013; Sultana *et al*., 2015; Mohsin *et al*., 2017), internal fruit quality characteristics (Grisales *et al*., 2015), flesh colour and carotenoid content (Itle and Kabelka, 2009), and plant and fruit morphology (Pandey *et al*., 2008; Du *et al*., 2011; Naik *et al*., 2015). However, no single study that includes a comprehensive list of morpho-agronomic, yield and internal fruit characteristics that relate specifically to the butternut type could be found in literature. Therefore, the aims of this study were to: (i) estimate the genetic parameters in Starke Ayres butternut germplasm in order to establish selection feasibility; and (ii) evaluate potential correlations between morpho-agronomic and internal fruit quality characteristics. Results obtained will contribute to the design of an effective and efficient crop improvement butternut-breeding programme.

## 5.3 Materials and methods

### 5.3.1 Plant material, site description and trial design

The material studied in this Chapter involved all the hybrids introduced in Chapter 3 and consisted of 27 $F_1$ genotypes. These genotypes included commercial hybrids from Starke Ayres and opposition companies and new hybrid combinations. The new hybrids were designed between parental lines selected on historical data confirming good general combining ability or above average *per se* performance. The $F_1$ genotypes were evaluated in three locations across South Africa *viz.* Jacobsdal, Kaalfontein and Oudtshoorn in the summers of 2018/2019 and 2019/2020. Each location within a specific season represented a unique environment (E) resulting in six different environments (E1 to E6). E1 and E2 represented Jacobsdal, E3 and E4 represented Kaalfontein and E5 and E6 represented Oudtshoorn for each of the two seasons respectively. Refer to Chapter 3, Sections 3.3.1 to 3.3.2 for detailed descriptions regarding the sites, trial design and management, and plant material used in this study.

### 5.3.2 Data collection and statistical analysis

Refer to Chapter 3, Section 3.3.3 for detailed descriptions regarding the collection of data on morpho-agronomic and internal fruit quality characteristics used in this study. The data collected were subjected to combined analyses of variance (ANOVA) across environments to establish the level of variability among the genotypes using Genstat® for Windows, 19$^{th}$ edition (VSN International, 2017). The ANOVA and partitioning of phenotypic variation for the characteristics within the environments were determined using the Variability package in R

(Popat *et al*., 2020). The phenotypic variance ($\sigma_p^2$) for each trait was partitioned into environmental variance ($\sigma_e^2$), genotype variance ($\sigma_g^2$) and genotype x environment (G x E) interaction variance ($\sigma_{gl}^2$) using the equations of Allard (1960) and Muhder *et al*. (2020):

$$\sigma_e^2 = MS_e$$

$$\sigma_g^2 = \frac{MS_g - MS_e}{lr}$$

$$\sigma_{gl}^2 = \frac{MS_{gl} - MS_e}{r}$$

$$\sigma_p^2 = \sigma_g^2 + \sigma_e^2 + \sigma_{gl}^2$$

Where, $MS_e$ is the residual mean square, $MS_g$ is the mean squares of the genotypes, $MS_{gl}$ is the mean squares of the G x E interaction, $r$ is the number of replications and $l$ is the number of environments.

To compare the variation amongst characteristics, the phenotypic coefficient of variation (PVC) the genotypic coefficient of variation (GCV) and the environmental coefficient of variation (ECV) were all computed using the equations of Burton (1952), Allard (1960) and Aruah *et al*. (2012) where $x$ is the grand mean of the characteristic:

$$PCV = \frac{\sqrt{\sigma_p^2}}{x} \times 100$$

$$GCV = \frac{\sqrt{\sigma_g^2}}{x} \times 100$$

$$ECV = \frac{\sqrt{\sigma_e^2}}{x} \times 100$$

The broad-sense heritability ($h_{bs}^2$) was computed using the flowing equation of Allard (1960) and Muhder *et al*. (2020):

$$h_{bs}^2 = \frac{\sigma_g^2}{\sigma_p^2}$$

Genetic advance (GA) is the improvement in the mean genotypic values of selected families, compared to the base population (Naik and Prasad, 2016). Both GA and GA as a percentage

of the mean (GAM) were computed using the equations of Johnson *et al*. (1955) and Muhder *et al.* (2020):

$$GA = k\sigma_p h_b^2$$

$$GAM = \frac{GA}{x} \times 100$$

Where $k$ is the selection intensity ($k$ = 2.06 at 5%) and $\sigma_p$ is the phenotypic standard deviation.

Phenotypic ($r_p$) and genotypic $(r_g)$ correlations and path analyses were calculated to estimate associations between different characteristics using the Variability package in R (Popat *et al.,* 2020) with reference to the formulae proposed by Singh and Chaudhary (1977):

$$r_p = \frac{PCOV_{XY}}{\sqrt{(PV_X.PV_Y)}}$$

$$r_g = \frac{GCOV_{XY}}{\sqrt{(GV_X.GV_Y)}}$$

Where $PCOV_{XY}$ is the phenotypic covariance between characteristics X and Y, $PV_X$ is the phenotypic variance of X, $PV_Y$ are the phenotypic variance of Y, $GCOV_{XY}$ is the genotypic covariance between characteristics X and Y, $GV_X$ is the genotypic variance of X and $PV_Y$ is the genotypic variance of Y.

Bartlett's test, which is used when there are more than two variances to be compared (Baye *et al*., 2020), confirmed that the variances among the environments were significantly different. The Stats package in R with reference to Bartlett (1937) was used. It was, therefore, inappropriate to conduct correlation and path coefficient analyses across combined environments and, as a result, each environment was analysed separately.

## 5.4 Results

### 5.4.1 Genetic variability

Data analysed for this Chapter were generated using a subset of 27 genotypes used in Chapter 3 and consisted of $F_1$ genotypes only. The data were subjected to an ANOVA across the six environments and this revealed highly significant (P≤0.001) differences among both the genotypes and the environments for all characteristics measured (Tables 5.1 to 5.3). When the yellow-blue colour contribution in the leaves (Leaf b*), leaf width (LW) and penetrometer

reading (PEN) as an indication of mesocarp firmness are excluded, significant G x E interaction effects were recorded for all characteristics.

Estimates of the genetic parameters, *viz.* $\sigma_p^2$, $\sigma_g^2$, $\sigma_e^2$, $\sigma_{gl}^2$, PCV, GCV, $h_{bs}^2$, GA and GAM, calculated from the mean square (MS) values from the ANOVA across the six environments are presented in Table 5.4. The genetic parameters calculated for the individual environments are presented in the Appendix (Tables A1 to A6). The computed PCV values for traits across environments ranged from -11.9 for the green-red colour contribution in the leaves (Leaf a*) to 34.0 for average fruit mass (AFM), while GCV ranged from -7.0 to 30.1, for the same characteristics (Table 5.4). The values of the PCV were generally higher than the corresponding GCV values, which confirmed the influence of environmental conditions. The only characteristics demonstrating high GCV estimates were AFM (30.1) and fruit number (FN) per plot (26.1), with the GCV estimates for chlorophyll content (CHL), Leaf a*, Leaf b*, LW, petiole length (PL) and dry matter yield (DMY) all calculated as low (<10). The remainder of the characteristics showed medium GCV values of between 10 and 20. The LW was the only characteristic with a low PCV (7.3), while high estimates were calculated for AFM (34.0), DMY (21.3), FN (31.7), yield (21.1) and the green-red colour contribution in the fruit mesocarp (Fruit a*) (21.3). The remaining traits showed moderate values between 10 and 20.

Although variation occurred across the different environments, high estimates for both PCV and GCV were observed for AFM and FN in all six individual environments (Appendix; Tables A1 to A6). A high $h_{bs}^2$ was estimated for AFM (0.79) using MS values across environments (Table 5.4) and this was also high for all six individual environments (ranging between 0.82 and 0.92) (Appendix; Tables A1 to A6). Moderately high $h_{bs}^2$ values were calculated for FN (0.68) and PEN (0.61) across environments but this pattern was not always consistently repeated within the individual environments. Using the across environments MS values, medium levels of $h_{bs}^2$ (ranging between 0.41 and 0.59) were calculated for CHL, Leaf b*, LW, PL, total soluble solids (TSS), dry matter content (DMC), Fruit a* and internal breakdown of the fruit mesocarp (IBD), while all remaining characteristics had low $h_{bs}^2$. Estimates for GAM ranged from 6.6% (LW) to 55.0% (AFM) and is an indication of the improvement that can be expected with regard to characteristics after one cycle of selection with a 5% selection intensity. Additional GAM estimates that were high included FN (44.3%) and Fruit a* (24.5%) and this was mostly in agreement with the individual environment analyses.

**Table 5.1** Combined analysis of variance showing mean squares of leaf chlorophyll content (CHL), green-red colour contribution in leaves (Leaf a*), yellow-blue colour contribution in leaves (Leaf b*), leaf width (LW) and petiole length (PL) across six environments

| Source of variation | df | CHL | Leaf a* | Leaf b* | LW | PL |
|---|---|---|---|---|---|---|
| Replication | 2 | 48.32 | 3.80 | 4.78 | 4509.80 | 8573.10 |
| Genotype (G) | 26 | 213.36 *** | 35.38 *** | 78.91 *** | 3779.20 *** | 16029.50 *** |
| Environment (E) | 5 | 2985.80 *** | 1337.76 *** | 1490.27 *** | 53071.00 *** | 141770.60 *** |
| G x E | 130 | 19.84 ** | 4.53 ** | 3.43 NS | 281.50 NS | 837.60 *** |
| Residual | 322 | 13.39 | 2.98 | 3.02 | 237.80 | 494.70 |
| Total | 485 | | | | | |

**P≤0.01, ***P≤0.001, NS: Not significant, df: Degrees of freedom

**Table 5.2** Combined analysis of variance showing mean squares of average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), fruit uniformity and yield across six environments

| Source of variation | df | AFM | DMY | FN | Uniformity | Yield |
|---|---|---|---|---|---|---|
| Replication | 2 | 0.01 | 19.21 | 354.94 | 32.10 | 532.40 |
| Genotype (G) | 26 | 5.03 *** | 12.53 *** | 3128.75 *** | 871.37 *** | 1629.80 *** |
| Environment (E) | 5 | 15.47 *** | 800.89 *** | 13286.47 *** | 1298.15 *** | 63231.50 *** |
| G x E | 130 | 0.16 *** | 3.01 ** | 120.61 *** | 122.59 *** | 297.10 *** |
| Residual | 322 | 0.03 | 1.97 | 60.65 | 60.26 | 166.80 |
| Total | 485 | | | | | |

**P≤0.01, ***P≤0.001, df: Degrees of freedom

**Table 5.3** Combined analysis of variance showing mean squares of total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) across six environments

| Source of variation | df | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|
| Replication | 2 | 0.03 | 7.40 | 19.44 | 658.46 | 0.24 |
| Genotype (G) | 26 | 17.61 *** | 26.08 *** | 140.46 *** | 1627.49 *** | 1.60 *** |
| Environment (E) | 5 | 30.45 *** | 46.77 *** | 146.09 *** | 2268.99 *** | 4.61 *** |
| G x E | 130 | 1.11 *** | 1.77 *** | 8.58 *** | 216.64 *** | 0.06 NS |
| Residual | 322 | 0.63 | 1.02 | 4.67 | 78.93 | 0.05 |
| Total | 485 | | | | | |

***P≤0.001, NS: Not significant, df: Degrees of freedom

**Table 5.4** Estimates of genetic parameters for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) across six environments

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Max | 67.13 | -9.39 | 35.59 | 370.00 | 561.00 | 4.25 | 19.79 | 120.00 | 80.00 | 170.12 | 12.65 | 16.25 | 26.02 | 90.00 | 4.07 |
| Min | 26.96 | -29.43 | 12.77 | 194.00 | 222.00 | 0.57 | 1.90 | 9.00 | 30.00 | 19.26 | 4.67 | 6.02 | 3.86 | 16.00 | 1.33 |
| Mean | 42.16 | -19.27 | 26.05 | 290.98 | 349.04 | 1.75 | 7.99 | 50.00 | 57.22 | 81.07 | 7.58 | 9.86 | 17.27 | 78.25 | 2.71 |
| SEM | 1.22 | 0.58 | 0.58 | 5.14 | 7.41 | 0.06 | 0.47 | 2.60 | 2.59 | 4.31 | 0.27 | 0.34 | 0.72 | 2.96 | 0.08 |
| CD 5% | 2.40 | 1.13 | 1.14 | 10.11 | 14.59 | 0.12 | 0.92 | 5.10 | 5.09 | 8.47 | 0.52 | 0.66 | 1.42 | 5.83 | 0.15 |
| $\sigma_e^2$ | 13.39 | 2.98 | 3.02 | 237.80 | 494.70 | 0.03 | 1.97 | 60.65 | 60.26 | 166.80 | 0.63 | 1.02 | 4.67 | 78.93 | 0.05 |
| $\sigma_g^2$ | 11.11 | 1.80 | 4.22 | 196.74 | 863.04 | 0.28 | 0.59 | 170.45 | 45.06 | 81.28 | 0.94 | 1.39 | 7.54 | 86.03 | 0.09 |
| $\sigma_{gl}^2$ | 2.15 | 0.52 | 0.14 | 14.57 | 114.30 | 0.04 | 0.35 | 19.99 | 20.78 | 43.43 | 0.16 | 0.25 | 1.30 | 45.90 | 0.00 |
| $\sigma_p^2$ | 26.65 | 5.30 | 7.37 | 449.11 | 1472.04 | 0.35 | 2.90 | 251.09 | 126.10 | 291.51 | 1.74 | 2.66 | 13.51 | 210.86 | 0.14 |
| ECV | 8.68 | -8.96 | 6.67 | 5.30 | 6.37 | 10.68 | 17.54 | 15.58 | 13.57 | 15.93 | 10.51 | 10.25 | 12.51 | 11.35 | 8.28 |
| GCV | 7.91 | -6.96 | 7.88 | 4.82 | 8.42 | 30.10 | 9.59 | 26.11 | 11.73 | 11.12 | 12.81 | 11.97 | 15.90 | 11.85 | 10.82 |
| PCV | 12.24 | -11.94 | 10.42 | 7.28 | 10.99 | 33.95 | 21.31 | 31.69 | 19.62 | 21.06 | 17.38 | 16.55 | 21.29 | 18.56 | 13.82 |
| $h_{bs}^2$ | 0.42 | 0.34 | 0.57 | 0.44 | 0.59 | 0.79 | 0.20 | 0.68 | 0.36 | 0.28 | 0.54 | 0.52 | 0.56 | 0.41 | 0.61 |
| GA | 4.43 | 1.61 | 3.20 | 19.12 | 46.34 | 0.96 | 0.71 | 22.16 | 8.27 | 9.81 | 1.47 | 1.76 | 4.23 | 12.20 | 0.47 |
| GAM | 10.52 | -8.36 | 12.28 | 6.57 | 13.28 | 54.99 | 8.89 | 44.32 | 14.45 | 12.10 | 19.46 | 17.82 | 24.48 | 15.60 | 17.45 |

Max: Maximum, Min: Minimum, SEM: Standard error of mean, CD: Critical difference, $\sigma_e^2$: Environmental variance, $\sigma_g^2$: Genotypic variance, $\sigma_{gl}^2$: Genotype x environment variance, $\sigma_p^2$: Phenotypic variance, ECV: Environmental coefficient of variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, $h_{bs}^2$: Broad-sense heritability, GA: Genetic advance, GAM: Genetic advance as a percentage of the mean

### 5.4.2 Correlation coefficients

Genotypic and phenotypic correlation coefficients were estimated between all morpho-agronomic and fruit quality characteristics for all six individual environments. The results (Tables 5.5 to 5.10) showed both highly significant (P≤0.01) and significant (P≤0.05) correlations at phenotypic and genotypic level. Positive and negative correlations were observed. Due to large residual MS values in the ANOVA for DMY in E1 and IBD in E4 (data not shown), genotypic correlations could not be calculated for these traits in these environments. Due to the large number of characteristics and environments included in this study, only correlations higher than 0.6 and recurring in at least three of the environments, were discussed. These are indicated in green in Tables 5.5 to 5.10.

The only consistently high and significant associations amongst plant characteristics included negative phenotypic and genotypic correlations between Leaf a* and Leaf b*. These correlations were stronger than -0.85 in E2 (Table 5.6), E3 (Table 5.7) and E4 (Table 5.8) and stronger than -0.65 in E1 (Table 5.5), E5 (Table 5.9) and E6 (Table 5.10). Negative genotypic correlations of -0.64, -0.72 and -0.81 were observed between Leaf a* and PL in E3, E4, E6, respectively.

Numerous strong associations between plant characteristics and yield components were observed across environments but genotypic correlations only between CHL and both AFM and FN recurred consistently. The genotypic correlations between CHL and AFM ranged from -0.52 to -0.78 with correlations in E1, E2, E5 and E6 stronger than -0.6 (Tables 5.5, 5.6, 5.9 and 5.10, respectively). These environments, as well as E3 (Table 5.7), showed positive correlations between CHL and FN ranging from 0.64 to as high as 0.87. No significant recurring correlations higher than 0.6 were observed between plant characteristics and internal fruit quality characteristics. Both AFM and FN demonstrated a strong association in all environments on both a phenotypic and a genotypic level, ranging from -0.69 to -0.81 and -0.71 to -0.96, respectively. Yield was also associated with various fruit quality characteristics on a genotypic level, but for some of the environments only.

Genotypic correlations for yield and TSS included -0.93 (E1), -0.79 (E3), -0.83 (E4) and -0.61 (E5); yield and DMC included -1.12 (E1), -0.79 (E3) and -0.87 (E4); and yield and Fruit a* included -0.74 (E1), -0.62 (E3) and -0.61 (E5). However, most of these genotypic correlations were not supported by phenotypic correlations. Correlations were over-estimated (in some cases higher than 1) in both E1 and E6, which was due to large residual MS values influencing the yield genetic variance (data not shown).

**Table 5.5** Phenotypic ($r_p$) and genotypic correlations ($r_g$) between chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) for Jacobsdal 2018/2019 (E1)

| | $r$ | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | P | 1.00** | 0.18 | -0.10 | -0.17 | 0.14 | -0.58** | -0.05 | 0.48** | -0.23* | -0.26* | 0.19 | 0.24* | 0.29** | 0.17 | 0.05 |
| | G | 1.00** | 0.28 | -0.10 | -0.28 | 0.20 | -0.76** | | 0.72** | -0.09 | -0.71** | 0.33 | 0.51** | 0.50** | 0.26 | 0.03 |
| Leaf a* | P | | 1.00** | -0.79** | -0.09 | -0.43** | -0.31** | 0.04 | 0.39** | 0.24* | 0.05 | -0.09 | -0.02 | 0.15 | 0.12 | 0.00 |
| | G | | 1.00** | -0.76** | -0.06 | -0.59** | -0.48* | | 0.45* | 0.55** | -0.48* | -0.21 | -0.01 | 0.36 | 0.02 | -0.03 |
| Leaf b* | P | | | 1.00** | -0.36** | 0.37** | 0.09 | -0.07 | -0.17 | -0.21 | -0.07 | 0.02 | 0.03 | -0.36** | -0.17 | 0.01 |
| | G | | | 1.00** | -0.53** | 0.47* | 0.13 | | -0.07 | -0.50** | 0.25 | 0.08 | 0.05 | -0.63** | -0.15 | 0.06 |
| LW | P | | | | 1.00** | 0.13 | 0.39** | -0.08 | -0.41** | 0.03 | -0.02 | 0.08 | -0.08 | 0.16 | 0.33** | 0.09 |
| | G | | | | 1.00** | 0.01 | 0.54** | | -0.60** | 0.11 | 0.11 | 0.34 | 0.11 | 0.42* | 0.48* | 0.10 |
| PL | P | | | | | 1.00** | 0.07 | -0.10 | -0.09 | -0.19 | -0.09 | 0.08 | 0.01 | -0.07 | 0.11 | -0.18 |
| | G | | | | | 1.00** | 0.10 | | -0.08 | -0.27 | 0.06 | 0.01 | -0.05 | -0.09 | 0.11 | -0.33 |
| AFM | P | | | | | | 1.00** | 0.12 | -0.81** | -0.04 | 0.41** | -0.17 | -0.33** | -0.39** | 0.19 | -0.04 |
| | G | | | | | | 1.00** | | -0.96** | -0.07 | 0.81** | -0.27 | -0.56** | -0.57** | 0.25 | -0.07 |
| DMY | P | | | | | | | 1.00** | 0.24* | 0.20 | 0.59** | 0.35** | 0.39** | 0.11 | -0.05 | 0.28* |
| | G | | | | | | | | | | | | | | | |
| FN | P | | | | | | | | 1.00** | 0.13 | 0.15 | -0.04 | 0.08 | 0.18 | -0.24* | -0.12 |
| | G | | | | | | | | 1.00** | 0.13 | -0.68** | 0.05 | 0.38 | 0.46* | -0.41* | -0.12 |
| Uniform | P | | | | | | | | | 1.00** | 0.16 | -0.06 | 0.01 | 0.12 | -0.15 | -0.08 |
| | G | | | | | | | | | 1.00** | 0.28 | -0.19 | -0.20 | 0.16 | -0.28 | -0.17 |
| Yield | P | | | | | | | | | | 1.00** | -0.41** | -0.50** | -0.39** | -0.14 | -0.30** |
| | G | | | | | | | | | | 1.00** | -0.93** | -1.12** | -0.74** | -0.31 | -0.68** |
| TSS | P | | | | | | | | | | | 1.00** | 0.86** | 0.42** | 0.18 | 0.68** |
| | G | | | | | | | | | | | 1.00** | 0.93** | 0.21 | 0.22 | 0.78** |
| DMC | P | | | | | | | | | | | | 1.00** | 0.50** | 0.14 | 0.69** |
| | G | | | | | | | | | | | | 1.00** | 0.33 | 0.18 | 0.86** |
| Fruit a* | P | | | | | | | | | | | | | 1.00** | 0.03 | 0.18 |
| | G | | | | | | | | | | | | | 1.00** | -0.05 | -0.08 |
| IBD | P | | | | | | | | | | | | | | 1.00** | 0.27* |
| | G | | | | | | | | | | | | | | 1.00** | 0.24 |
| PEN | P | | | | | | | | | | | | | | | 1.00** |
| | G | | | | | | | | | | | | | | | 1.00** |

*P≤0.05, **P≤0.01, G: Genotypic correlation, P: Phenotypic correlation; Correlations higher than 0.6 and recurring in at least three of the environments are highlighted in green

**Table 5.6** Phenotypic ($r_p$) and genotypic correlations ($r_g$) between chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) for Jacobsdal 2019/2020 (E2)

| | $r$ | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | P | 1.00** | 0.23* | -0.16 | -0.06 | -0.10 | -0.60** | 0.35** | 0.62** | 0.10 | 0.16 | 0.29** | 0.33** | 0.37** | -0.11 | 0.32** |
| | G | 1.00** | 0.41* | -0.26 | -0.15 | -0.21 | -0.66** | 0.42* | 0.73** | 0.21 | 0.22 | 0.35 | 0.38 | 0.48* | -0.12 | 0.41* |
| Leaf a* | P | | 1.00** | -0.85** | 0.21 | -0.26* | -0.48** | -0.11 | 0.28** | 0.02 | -0.13 | -0.04 | -0.04 | 0.05 | 0.11 | -0.11 |
| | G | | 1.00** | -0.87** | 0.20 | -0.44* | -0.68** | -0.07 | 0.50** | 0.03 | -0.05 | -0.19 | -0.14 | 0.06 | 0.17 | -0.18 |
| Leaf b* | P | | | 1.00** | -0.45** | 0.33** | 0.38** | 0.35** | -0.05 | 0.01 | 0.35** | 0.07 | 0.07 | -0.12 | -0.18 | 0.20 |
| | G | | | 1.00** | -0.56** | 0.52** | 0.52** | 0.46* | -0.11 | 0.03 | 0.43* | 0.19 | 0.17 | -0.13 | -0.27 | 0.29 |
| LW | P | | | | 1.00** | -0.14 | 0.09 | -0.38** | -0.35** | -0.22 | -0.46** | 0.19 | 0.11 | 0.07 | 0.37** | 0.09 |
| | G | | | | 1.00** | -0.34 | 0.15 | -0.57** | -0.50** | -0.26 | -0.70** | 0.19 | 0.14 | 0.11 | 0.65** | 0.11 |
| PL | P | | | | | 1.00** | 0.18 | 0.22* | -0.02 | -0.09 | 0.13 | 0.23* | 0.22* | -0.01 | 0.05 | 0.01 |
| | G | | | | | 1.00** | 0.24 | 0.29 | -0.05 | -0.27 | 0.15 | 0.35 | 0.32 | 0.03 | 0.06 | 0.03 |
| AFM | P | | | | | | 1.00** | -0.10 | -0.69** | -0.02 | 0.08 | -0.21 | -0.26* | -0.45** | 0.07 | 0.07 |
| | G | | | | | | 1.00** | -0.13 | -0.71** | -0.11 | 0.09 | -0.27 | -0.33 | -0.59** | 0.05 | 0.04 |
| DMY | P | | | | | | | 1.00** | 0.66** | -0.07 | 0.83** | 0.33** | 0.37** | 0.15 | 0.03 | 0.50** |
| | G | | | | | | | 1.00** | 0.68** | -0.17 | 0.85** | 0.35 | 0.39* | 0.10 | 0.05 | 0.58** |
| FN | P | | | | | | | | 1.00** | 0.00 | 0.61** | 0.05 | 0.10 | 0.13 | -0.09 | 0.12 |
| | G | | | | | | | | 1.00** | 0.01 | 0.58** | 0.15 | 0.20 | 0.22 | -0.13 | 0.19 |
| Uniform | P | | | | | | | | | 1.00** | 0.09 | -0.19 | -0.22 | -0.04 | -0.30** | -0.09 |
| | G | | | | | | | | | 1.00** | 0.03 | -0.25 | -0.32 | -0.17 | -0.67** | -0.05 |
| Yield | P | | | | | | | | | | 1.00** | -0.21 | -0.20 | -0.28* | -0.12 | 0.18 |
| | G | | | | | | | | | | 1.00** | -0.17 | -0.14 | -0.34 | -0.25 | 0.27 |
| TSS | P | | | | | | | | | | | 1.00** | 0.97** | 0.68** | 0.33** | 0.61** |
| | G | | | | | | | | | | | 1.00** | 1.00** | 0.72** | 0.56** | 0.68** |
| DMC | P | | | | | | | | | | | | 1.00** | 0.75** | 0.30** | 0.61** |
| | G | | | | | | | | | | | | 1.00** | 0.78** | 0.52** | 0.66** |
| Fruit a* | P | | | | | | | | | | | | | 1.00** | 0.04 | 0.33** |
| | G | | | | | | | | | | | | | 1.00** | 0.18 | 0.31 |
| IBD | P | | | | | | | | | | | | | | 1.00** | 0.19 |
| | G | | | | | | | | | | | | | | 1.00** | 0.34 |
| PEN | P | | | | | | | | | | | | | | | 1.00** |
| | G | | | | | | | | | | | | | | | 1.00** |

*P≤0.05, **P≤0.01, G: Genotypic correlation, P: Phenotypic correlation; Correlations higher than 0.6 and recurring in at least three of the environments are highlighted in green

**Table 5.7** Phenotypic ($r_p$) and genotypic correlations ($r_g$) between chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) for Kaalfontein 2018/2019 (E3)

| | $r$ | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | P | 1.00** | 0.08 | -0.15 | -0.07 | 0.02 | -0.39** | 0.19 | 0.49** | 0.13 | -0.02 | 0.19 | 0.16 | 0.05 | 0.03 | 0.18 |
| | G | 1.00** | 0.22 | -0.33 | -0.22 | -0.01 | -0.52** | 0.56** | 0.64** | 0.22 | -0.01 | 0.39* | 0.33 | 0.03 | -0.19 | 0.47* |
| Leaf a* | P | | 1.00** | -0.94** | 0.22 | -0.54** | 0.08 | -0.18 | -0.15 | 0.10 | -0.16 | -0.03 | -0.07 | 0.07 | 0.05 | -0.01 |
| | G | | 1.00** | -1.00** | 0.26 | -0.64** | 0.04 | -0.27 | -0.13 | 0.19 | -0.17 | -0.04 | -0.09 | 0.10 | 0.20 | -0.07 |
| Leaf b* | P | | | 1.00** | -0.21 | 0.42** | -0.05 | 0.10 | 0.12 | -0.08 | 0.14 | -0.05 | 0.01 | -0.09 | -0.08 | 0.00 |
| | G | | | 1.00** | -0.21 | 0.60** | 0.04 | 0.29 | 0.06 | -0.23 | 0.21 | 0.01 | 0.07 | -0.07 | -0.24 | 0.11 |
| LW | P | | | | 1.00** | 0.03 | 0.38** | 0.10 | -0.46** | -0.13 | -0.13 | 0.26* | 0.23* | 0.19 | 0.31** | 0.10 |
| | G | | | | 1.00** | -0.11 | 0.43* | 0.18 | -0.59** | -0.14 | -0.25 | 0.42* | 0.40* | 0.26 | 0.49** | 0.22 |
| PL | P | | | | | 1.00** | 0.20 | 0.21 | -0.05 | -0.26* | 0.32** | -0.09 | -0.10 | -0.50** | 0.21 | -0.27* |
| | G | | | | | 1.00** | 0.24 | 0.26 | -0.10 | -0.39* | 0.28 | -0.11 | -0.11 | -0.67** | 0.42* | -0.38* |
| AFM | P | | | | | | 1.00** | 0.24* | -0.79** | -0.19 | 0.45** | -0.23* | -0.22* | -0.35** | 0.14 | -0.16 |
| | G | | | | | | 1.00** | 0.19 | -0.82** | -0.29 | 0.52** | -0.42* | -0.41* | -0.46* | 0.34 | -0.34 |
| DMY | P | | | | | | | 1.00** | 0.03 | -0.01 | 0.46** | 0.42** | 0.45** | 0.10 | 0.07 | 0.43** |
| | G | | | | | | | 1.00** | 0.13 | -0.07 | 0.45* | 0.16 | 0.18 | -0.14 | 0.16 | 0.57** |
| FN | P | | | | | | | | 1.00** | 0.26* | 0.12 | -0.11 | -0.11 | 0.11 | -0.30** | 0.10 |
| | G | | | | | | | | 1.00** | 0.44* | 0.04 | 0.02 | 0.01 | 0.17 | -0.61** | 0.29 |
| Uniform | P | | | | | | | | | 1.00** | 0.06 | -0.05 | -0.06 | 0.08 | -0.23* | 0.12 |
| | G | | | | | | | | | 1.00** | 0.15 | -0.13 | -0.16 | 0.05 | -0.61** | 0.28 |
| Yield | P | | | | | | | | | | 1.00** | -0.57** | -0.57** | -0.48** | -0.22* | -0.18 |
| | G | | | | | | | | | | 1.00** | -0.79** | -0.79** | -0.62** | -0.36 | -0.20 |
| TSS | P | | | | | | | | | | | 1.00** | 0.97** | 0.51** | 0.24* | 0.60** |
| | G | | | | | | | | | | | 1.00** | 0.99** | 0.51** | 0.46* | 0.66** |
| DMC | P | | | | | | | | | | | | 1.00** | 0.56** | 0.26* | 0.61** |
| | G | | | | | | | | | | | | 1.00** | 0.56** | 0.45* | 0.66** |
| Fruit a* | P | | | | | | | | | | | | | 1.00** | -0.03 | 0.37** |
| | G | | | | | | | | | | | | | 1.00** | -0.18 | 0.37 |
| IBD | P | | | | | | | | | | | | | | 1.00** | 0.00 |
| | G | | | | | | | | | | | | | | 1.00** | -0.17 |
| PEN | P | | | | | | | | | | | | | | | 1.00** |
| | G | | | | | | | | | | | | | | | 1.00** |

*P≤0.05, **P≤0.01, G: Genotypic correlation, P: Phenotypic correlation; Correlations higher than 0.6 and recurring in at least three of the environments are highlighted in green

**Table 5.8** Phenotypic ($r_p$) and genotypic correlations ($r_g$) between chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) for Kaalfontein 2019/2020 (E4)

| | $r$ | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | P | 1.00** | 0.28* | -0.42** | -0.02 | -0.25* | -0.46** | -0.08 | 0.44** | 0.14 | -0.22* | 0.13 | 0.14 | 0.30** | -0.12 | 0.18 |
| | G | 1.00** | 0.41* | -0.50** | -0.09 | -0.28 | -0.57** | -0.22 | 0.54** | 0.27 | -0.33 | 0.14 | 0.18 | 0.44* | | 0.22 |
| Leaf a* | P | | 1.00** | -0.93** | 0.13 | -0.39** | -0.09 | -0.32** | -0.13 | 0.12 | -0.27* | 0.03 | -0.01 | -0.02 | -0.02 | 0.00 |
| | G | | 1.00** | -0.97** | 0.12 | -0.72** | -0.12 | -0.53** | -0.20 | 0.29 | -0.42* | 0.21 | 0.10 | 0.12 | | -0.03 |
| Leaf b* | P | | | 1.00** | -0.17 | 0.38** | 0.10 | 0.24* | 0.08 | -0.10 | 0.32** | -0.15 | -0.10 | -0.02 | 0.04 | -0.06 |
| | G | | | 1.00** | -0.22 | 0.56** | 0.15 | 0.55** | 0.13 | -0.08 | 0.56** | -0.31 | -0.21 | -0.17 | | -0.04 |
| LW | P | | | | 1.00** | 0.19 | 0.42** | 0.09 | -0.39** | -0.23* | -0.20 | 0.41** | 0.29** | -0.17 | 0.22 | 0.17 |
| | G | | | | 1.00** | 0.16 | 0.60** | 0.32 | -0.55** | -0.59** | -0.27 | 0.56** | 0.43* | -0.27 | | 0.28 |
| PL | P | | | | | 1.00** | 0.48** | 0.22 | -0.15 | -0.15 | 0.25* | -0.08 | -0.10 | -0.47** | 0.17 | -0.04 |
| | G | | | | | 1.00** | 0.58** | 0.44* | -0.19 | -0.30 | 0.32 | -0.12 | -0.12 | -0.58** | | -0.03 |
| AFM | P | | | | | | 1.00** | 0.20 | -0.75** | -0.14 | 0.21 | 0.02 | -0.06 | -0.52** | 0.17 | 0.00 |
| | G | | | | | | 1.00** | 0.27 | -0.80** | -0.24 | 0.19 | 0.03 | -0.06 | -0.61** | | 0.05 |
| DMY | P | | | | | | | 1.00** | 0.18 | -0.04 | 0.56** | 0.27* | 0.27* | -0.10 | -0.08 | 0.30** |
| | G | | | | | | | 1.00** | -0.03 | -0.87** | 0.38* | 0.17 | 0.10 | -0.28 | | 0.49* |
| FN | P | | | | | | | | 1.00** | 0.06 | 0.36** | -0.32** | -0.28* | 0.16 | -0.18 | -0.10 |
| | G | | | | | | | | 1.00** | 0.00 | 0.29 | -0.42* | -0.36 | 0.23 | | -0.19 |
| Uniform | P | | | | | | | | | 1.00** | -0.02 | -0.10 | 0.00 | 0.11 | -0.18 | 0.00 |
| | G | | | | | | | | | 1.00** | -0.18 | -0.32 | -0.17 | 0.12 | | -0.14 |
| Yield | P | | | | | | | | | | 1.00** | -0.59** | -0.63** | -0.49** | -0.12 | -0.23* |
| | G | | | | | | | | | | 1.00** | -0.83** | -0.87** | -0.57** | | -0.35 |
| TSS | P | | | | | | | | | | | 1.00** | 0.96** | 0.38** | 0.08 | 0.56** |
| | G | | | | | | | | | | | 1.00** | 0.98** | 0.38* | | 0.65** |
| DMC | P | | | | | | | | | | | | 1.00** | 0.49** | 0.05 | 0.57** |
| | G | | | | | | | | | | | | 1.00** | 0.49** | | 0.64** |
| Fruit a* | P | | | | | | | | | | | | | 1.00** | -0.09 | 0.11 |
| | G | | | | | | | | | | | | | 1.00** | | 0.13 |
| IBD | P | | | | | | | | | | | | | | 1.00** | 0.08 |
| | G | | | | | | | | | | | | | | | |
| PEN | P | | | | | | | | | | | | | | | 1.00** |
| | G | | | | | | | | | | | | | | | 1.00** |

*P≤0.05, **P≤0.01, G: Genotypic correlation, P: Phenotypic correlation; Correlations higher than 0.6 and recurring in at least three of the environments are highlighted in green

**Table 5.9** Phenotypic ($r_p$) and genotypic correlations ($r_g$) between chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) for Oudtshoorn 2018/2019 (E5)

| | $r$ | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | P | 1.00** | 0.43** | -0.49** | -0.09 | -0.39** | -0.41** | 0.02 | 0.42** | 0.11 | -0.09 | 0.10 | 0.14 | 0.26* | 0.04 | 0.02 |
| | G | 1.00** | 0.42* | -0.60** | 0.06 | -0.47* | -0.78** | 0.17 | 0.87** | 0.33 | -0.13 | 0.23 | 0.31 | 0.74** | -0.06 | 0.10 |
| Leaf a* | P | | 1.00** | -0.78** | -0.13 | -0.09 | -0.21 | -0.14 | 0.12 | 0.03 | -0.20 | 0.08 | 0.08 | 0.17 | 0.28* | 0.16 |
| | G | | 1.00** | -0.65** | 0.03 | -0.10 | -0.30 | -0.28 | 0.21 | 0.01 | -0.32 | 0.04 | 0.08 | 0.44* | 0.51** | 0.22 |
| Leaf b* | P | | | 1.00** | -0.27* | 0.27* | 0.14 | 0.24* | -0.07 | -0.14 | 0.26* | -0.02 | -0.01 | -0.26* | -0.39** | -0.02 |
| | G | | | 1.00** | -0.73** | 0.38* | 0.21 | 0.43* | -0.15 | -0.24 | 0.38* | 0.06 | 0.02 | -0.59** | -0.60** | 0.01 |
| LW | P | | | | 1.00** | -0.06 | 0.35** | -0.21 | -0.43** | 0.09 | -0.28** | 0.04 | 0.02 | 0.19 | 0.29** | -0.02 |
| | G | | | | 1.00** | -0.32 | 0.47* | -0.28 | -0.56** | 0.06 | -0.46* | 0.21 | 0.16 | 0.43* | 0.49* | 0.05 |
| PL | P | | | | | 1.00** | -0.08 | 0.10 | 0.05 | -0.19 | 0.07 | 0.02 | 0.04 | -0.07 | -0.07 | -0.18 |
| | G | | | | | 1.00** | -0.09 | 0.12 | 0.05 | -0.29 | 0.07 | 0.06 | 0.03 | -0.14 | -0.07 | -0.24 |
| AFM | P | | | | | | 1.00** | -0.05 | -0.80** | -0.17 | 0.11 | -0.14 | -0.20 | -0.30** | 0.19 | 0.05 |
| | G | | | | | | 1.00** | -0.05 | -0.88** | -0.26 | 0.17 | -0.19 | -0.25 | -0.41* | 0.21 | 0.03 |
| DMY | P | | | | | | | 1.00** | 0.34** | 0.00 | 0.59** | 0.48** | 0.49** | 0.21 | -0.07 | 0.39** |
| | G | | | | | | | 1.00** | 0.18 | -0.04 | 0.44* | 0.44* | 0.49** | 0.07 | -0.01 | 0.49** |
| FN | P | | | | | | | | 1.00** | 0.21 | 0.40** | -0.08 | -0.03 | 0.07 | -0.28* | -0.18 |
| | G | | | | | | | | 1.00** | 0.34 | 0.21 | -0.10 | -0.01 | 0.12 | -0.37 | -0.19 |
| Uniform | P | | | | | | | | | 1.00** | 0.19 | -0.21 | -0.21 | -0.04 | -0.13 | -0.22* |
| | G | | | | | | | | | 1.00** | 0.30 | -0.32 | -0.34 | -0.10 | -0.15 | -0.28 |
| Yield | P | | | | | | | | | | 1.00** | -0.40** | -0.40** | -0.40** | -0.18 | -0.24* |
| | G | | | | | | | | | | 1.00** | -0.61** | -0.56** | -0.61** | -0.28 | -0.34 |
| TSS | P | | | | | | | | | | | 1.00** | 0.97** | 0.66** | 0.09 | 0.67** |
| | G | | | | | | | | | | | 1.00** | 1.00** | 0.68** | 0.27 | 0.77** |
| DMC | P | | | | | | | | | | | | 1.00** | 0.68** | 0.08 | 0.69** |
| | G | | | | | | | | | | | | 1.00** | 0.67** | 0.24 | 0.78** |
| Fruit a* | P | | | | | | | | | | | | | 1.00** | 0.07 | 0.32** |
| | G | | | | | | | | | | | | | 1.00** | 0.23 | 0.26 |
| IBD | P | | | | | | | | | | | | | | 1.00** | 0.24* |
| | G | | | | | | | | | | | | | | 1.00** | 0.37 |
| PEN | P | | | | | | | | | | | | | | | 1.00** |
| | G | | | | | | | | | | | | | | | 1.00** |

*P≤0.05, **P≤0.01, G: Genotypic correlation, P: Phenotypic correlation; Correlations higher than 0.6 and recurring in at least three of the environments are highlighted in green

**Table 5.10** Phenotypic ($r_p$) and genotypic correlations ($r_g$) between Chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) for Oudtshoorn 2019/2020 (E6)

| | $r$ | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | P | 1.00** | 0.29** | -0.30** | 0.01 | -0.04 | -0.44** | 0.17 | 0.49** | 0.06 | 0.10 | 0.13 | 0.09 | 0.24* | 0.19 | 0.16 |
| | G | 1.00** | 0.34 | -0.32 | -0.01 | -0.10 | -0.78** | 0.09 | 0.85** | 0.11 | -0.11 | 0.27 | 0.24 | 0.43* | 0.42* | 0.16 |
| Leaf a* | P | | 1.00** | -0.74** | -0.13 | -0.48** | -0.39** | -0.31** | 0.16 | 0.28* | -0.26* | -0.13 | -0.17 | 0.00 | 0.10 | -0.16 |
| | G | | 1.00** | -0.85** | 0.06 | -0.81** | -0.91** | -0.83** | 0.43* | 0.61** | -1.08** | -0.13 | -0.20 | 0.19 | 0.14 | -0.35 |
| Leaf b* | P | | | 1.00** | -0.31** | 0.29** | 0.21 | 0.36** | -0.02 | -0.33** | 0.28* | 0.16 | 0.23* | 0.06 | -0.24* | 0.06 |
| | G | | | 1.00** | -0.53** | 0.55** | 0.34 | 0.73** | 0.01 | -0.46* | 0.94** | 0.07 | 0.20 | -0.04 | -0.35 | 0.05 |
| LW | P | | | | 1.00** | 0.38** | 0.35** | -0.01 | -0.22* | -0.02 | 0.04 | -0.10 | -0.11 | -0.04 | 0.21 | 0.19 |
| | G | | | | 1.00** | -0.10 | 0.48* | -0.34 | -0.63** | 0.03 | -0.54** | 0.07 | -0.01 | -0.03 | 0.57** | 0.26 |
| PL | P | | | | | 1.00** | 0.39** | 0.32** | -0.10 | -0.06 | 0.31** | 0.02 | 0.06 | -0.03 | 0.04 | 0.02 |
| | G | | | | | 1.00** | 0.54** | 0.37 | -0.40* | -0.22 | 0.23 | 0.31 | 0.34 | 0.07 | 0.04 | -0.09 |
| AFM | P | | | | | | 1.00** | 0.22 | -0.72** | -0.19 | 0.32** | -0.15 | -0.11 | -0.32** | 0.06 | 0.22* |
| | G | | | | | | 1.00** | 0.20 | -0.89** | -0.29 | 0.35 | -0.06 | -0.02 | -0.42* | 0.11 | 0.23 |
| DMY | P | | | | | | | 1.00** | 0.36** | -0.23* | 0.83** | 0.36** | 0.42** | 0.21 | 0.26* | 0.50** |
| | G | | | | | | | 1.00** | 0.13 | -0.51** | 0.79** | 0.82** | 0.85** | 0.27 | 0.51** | 0.63** |
| FN | P | | | | | | | | 1.00** | 0.08 | 0.37** | 0.02 | 0.02 | 0.18 | 0.02 | -0.04 |
| | G | | | | | | | | 1.00** | 0.06 | 0.05 | 0.19 | 0.16 | 0.36 | 0.02 | -0.08 |
| Uniform | P | | | | | | | | | 1.00** | -0.19 | -0.12 | -0.13 | -0.13 | 0.10 | -0.17 |
| | G | | | | | | | | | 1.00** | -0.66** | -0.08 | -0.12 | -0.18 | 0.12 | -0.22 |
| Yield | P | | | | | | | | | | 1.00** | -0.18 | -0.14 | -0.19 | 0.13 | 0.24* |
| | G | | | | | | | | | | 1.00** | 0.27 | 0.32 | -0.18 | 0.31 | 0.33 |
| TSS | P | | | | | | | | | | | 1.00** | 0.97** | 0.69** | 0.28* | 0.49** |
| | G | | | | | | | | | | | 1.00** | 1.00** | 0.67** | 0.51** | 0.68** |
| DMC | P | | | | | | | | | | | | 1.00** | 0.68** | 0.27* | 0.52** |
| | G | | | | | | | | | | | | 1.00** | 0.62** | 0.49** | 0.69** |
| Fruit a* | P | | | | | | | | | | | | | 1.00** | -0.03 | 0.09 |
| | G | | | | | | | | | | | | | 1.00** | 0.06 | 0.02 |
| IBD | P | | | | | | | | | | | | | | 1.00** | 0.53** |
| | G | | | | | | | | | | | | | | 1.00** | 0.70** |
| PEN | P | | | | | | | | | | | | | | | 1.00** |
| | G | | | | | | | | | | | | | | | 1.00** |

G: Genotypic correlation, P: Phenotypic correlation, *P≤0.05, **P≤0.01; Correlations higher than 0.6 and recurring in at least three of the environments are highlighted in green

Associations amongst internal fruit quality characteristics were most prevalent. Both TSS and DMC had significant and positive correlations higher than 0.85 across all six environments, both on phenotypic and on genotypic level. There were significant and positive genotypic correlations higher than 0.65 between TSS and PEN across all environments, supported with phenotypic correlations higher than or equal to 0.60 in three of the environments. Similarly, positive genotypic correlations higher than 0.64 were observed between DMC and PEN, supported with phenotypic correlations higher than 0.60 in four of the environments. There were also positive correlations higher than 0.60 between DMC and Fruit a*, both on a phenotypic and on a genotypic level in three of the environments. Positive correlations both on genotypic and phenotypic level were also observed at three locations between TSS and Fruit a*.

### 5.4.3 Path coefficient analysis

Since DMY is a product of yield, DMY was excluded from the genotypic path coefficient analyses. Genotypic variance for IBD in E4 was estimated to be zero (Appendix; Table A4); therefore, a path coefficient analysis could not be conducted for this environment. The direct and indirect effects on yield of the 13 characteristics evaluated were estimated for E1, E2, E3, E5 and E6, and included both positive and negative effects (Tables 5.11 to 5.15). An analysis across all locations was not appropriate due to significantly different variances estimated for the individual environments. Direct and indirect effects contributing more than 20% of the partitioned genotypic correlation are indicated in green in each table.

In E1 (Table 5.11), the highest positive direct contributor towards yield was IBD (2.13), followed by FN (1.86) and uniformity (1.26). Strong negative direct effects included Leaf a* (-4.84), Leaf b* (-3.81), LW (-2.07) and CHL (-1.83). Various characteristics had a meaningful indirect effect on yield via Leaf a* and Leaf b*. In E2 (Table 5.12), excluding DMC (-2.15) and LW (-0.71), all direct effects on yield were positive. The strongest direct effects on yield included IBD (0.92) and Fruit a* (0.90). The strong indirect effects on yield were generally via DMC and IBD. DMC (1.25), AFM (1.21) and FN (0.97) had strong positive direct effects on yield in E3 (Table 5.13). The strongest negative effects on yield were due to TSS (-1.18), Fruit a* (-0.43) and IBD (-0.38). Various characteristics had noteworthy indirect effects on yield via AFM, FN, TSS and DMC. In E5 (Table 5.14), estimated effects were much higher compared to the other environments, confirming why path coefficient analysis could not be conducted across environments. The characteristics with the strongest positive direct effects on yield were TSS (11.94), PEN (6.75) and Fruit a* (4.84). The strongest negative direct effects on yield were due to DMC (-21.19), Leaf a* (-3.89) and LW (-2.09). Characteristics generally had indirect effects on yield via DMC and TSS.

**Table 5.11** Partitioning of genotypic correlations into direct (bold) and indirect effects of Chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) on yield in Jacobsdal 2018/2019 (E1)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | FN | Uniform | TSS | DMC | Fruit a* | IBD | PEN | Yield $r_g$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | **-1.832** | -1.366 | 0.383 | 0.589 | -0.145 | 0.270 | 1.339 | -0.114 | -0.004 | 0.126 | -0.485 | 0.554 | -0.030 | -0.71** |
| Leaf a* | -0.517 | **-4.842** | 2.901 | 0.123 | 0.426 | 0.170 | 0.843 | 0.693 | 0.003 | -0.001 | -0.350 | 0.047 | 0.023 | -0.48* |
| Leaf b* | 0.184 | 3.684 | **-3.813** | 1.092 | -0.340 | -0.045 | -0.130 | -0.630 | -0.001 | 0.012 | 0.608 | -0.314 | -0.053 | 0.25 |
| LW | 0.521 | 0.286 | 2.010 | **-2.073** | -0.006 | -0.192 | -1.125 | 0.140 | -0.004 | 0.027 | -0.408 | 1.033 | -0.094 | 0.11 |
| PL | -0.368 | 2.860 | -1.797 | -0.018 | **-0.721** | -0.036 | -0.142 | -0.334 | 0.000 | -0.013 | 0.089 | 0.234 | 0.308 | 0.06 |
| AFM | 1.385 | 2.308 | -0.480 | -1.117 | -0.073 | **-0.357** | -1.782 | -0.091 | 0.003 | -0.139 | 0.551 | 0.533 | 0.064 | 0.81** |
| FN | -1.317 | -2.191 | 0.266 | 1.252 | 0.055 | 0.341 | **1.862** | 0.170 | -0.001 | 0.093 | -0.446 | -0.874 | 0.113 | -0.68** |
| Uniform | 0.166 | -2.662 | 1.907 | -0.230 | 0.191 | 0.026 | 0.251 | **1.260** | 0.002 | -0.049 | -0.151 | -0.592 | 0.158 | 0.28 |
| TSS | -0.611 | 1.002 | -0.295 | -0.715 | -0.004 | 0.096 | 0.085 | -0.242 | **-0.013** | 0.228 | -0.205 | 0.471 | -0.723 | -0.93** |
| DMC | -0.933 | 0.028 | -0.181 | -0.224 | 0.037 | 0.201 | 0.704 | -0.250 | -0.012 | **0.247** | -0.320 | 0.388 | -0.802 | -1.12** |
| Fruit a* | -0.917 | -1.750 | 2.398 | -0.874 | 0.067 | 0.203 | 0.858 | 0.197 | -0.003 | 0.082 | **-0.967** | -0.110 | 0.076 | -0.74** |
| IBD | -0.476 | -0.107 | 0.562 | -1.005 | -0.079 | -0.089 | -0.763 | -0.350 | -0.003 | 0.045 | 0.050 | **2.132** | -0.225 | -0.31 |
| PEN | -0.058 | 0.121 | -0.217 | -0.209 | 0.239 | 0.025 | -0.226 | -0.213 | -0.010 | 0.212 | 0.079 | 0.514 | **-0.933** | -0.68** |
| Residual | -0.613 | | | | | | | | | | | | | |

*P≤0.05, **P≤0.01, Yield $r_g$: Yield genotypic correlation; Direct and indirect effects contributing more than 20% of the partitioned genotypic correlation are highlited in green

**Table 5.12** Partitioning of genotypic correlations into direct (bold) and indirect effects of Chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) on yield in Jacobsdal 2019/2020 (E2)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | FN | Uniform | TSS | DMC | Fruit a* | IBD | PEN | Yield $r_g$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | **0.142** | 0.126 | -0.132 | 0.109 | -0.028 | -0.397 | 0.446 | 0.030 | 0.208 | -0.807 | 0.433 | -0.109 | 0.199 | 0.22 |
| Leaf a* | 0.058 | **0.311** | -0.435 | -0.139 | -0.059 | -0.408 | 0.310 | 0.005 | -0.111 | 0.302 | 0.051 | 0.153 | -0.087 | -0.05 |
| Leaf b* | -0.037 | -0.269 | **0.502** | 0.397 | 0.070 | 0.310 | -0.066 | 0.004 | 0.115 | -0.366 | -0.121 | -0.252 | 0.143 | 0.43* |
| LW | -0.022 | 0.061 | -0.280 | **-0.711** | -0.046 | 0.092 | -0.310 | -0.038 | 0.113 | -0.305 | 0.103 | 0.597 | 0.052 | -0.70** |
| PL | -0.029 | -0.137 | 0.262 | 0.242 | **0.135** | 0.141 | -0.033 | -0.040 | 0.210 | -0.698 | 0.030 | 0.051 | 0.015 | 0.15 |
| AFM | -0.094 | -0.212 | 0.261 | -0.110 | 0.032 | **0.597** | -0.437 | -0.016 | -0.162 | 0.701 | -0.535 | 0.048 | 0.018 | 0.09 |
| FN | 0.103 | 0.157 | -0.054 | 0.359 | -0.007 | -0.425 | **0.615** | 0.001 | 0.087 | -0.427 | 0.195 | -0.116 | 0.094 | 0.58** |
| Uniform | 0.029 | 0.010 | 0.014 | 0.187 | -0.037 | -0.066 | 0.005 | **0.146** | -0.151 | 0.681 | -0.153 | -0.614 | -0.025 | 0.03 |
| TSS | 0.050 | -0.058 | 0.097 | -0.136 | 0.048 | -0.163 | 0.090 | -0.037 | **0.594** | -2.142 | 0.651 | 0.510 | 0.331 | -0.17 |
| DMC | 0.053 | -0.044 | 0.085 | -0.101 | 0.044 | -0.195 | 0.122 | -0.046 | 0.592 | **-2.150** | 0.704 | 0.477 | 0.322 | -0.14 |
| Fruit a* | 0.068 | 0.018 | -0.067 | -0.081 | 0.004 | -0.354 | 0.133 | -0.025 | 0.429 | -1.679 | **0.902** | 0.163 | 0.150 | -0.34 |
| IBD | -0.017 | 0.052 | -0.138 | -0.463 | 0.007 | 0.031 | -0.078 | -0.098 | 0.330 | -1.118 | 0.161 | **0.917** | 0.166 | -0.25 |
| PEN | 0.058 | -0.055 | 0.146 | -0.075 | 0.004 | 0.022 | 0.118 | -0.007 | 0.401 | -1.415 | 0.276 | 0.311 | **0.490** | 0.27 |
| Residual: | 0.043 | | | | | | | | | | | | | |

*P≤0.05, **P≤0.01, Yield $r_g$: Yield genotypic correlation; Direct and indirect effects contributing more than 20% of the partitioned genotypic correlation are highlited in green

**Table 5.13** Partitioning of genotypic correlations into direct (bold) and indirect effects of Chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) on yield in Kaalfontein 2018/2019 (E3)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | FN | Uniform | TSS | DMC | Fruit a* | IBD | PEN | Yield $r_g$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | **0.026** | 0.039 | -0.034 | -0.017 | 0.000 | -0.629 | 0.619 | -0.019 | -0.464 | 0.414 | -0.011 | 0.072 | -0.011 | -0.01 |
| Leaf a* | 0.006 | **0.178** | -0.104 | 0.020 | 0.015 | 0.046 | -0.124 | -0.016 | 0.048 | -0.115 | -0.045 | -0.078 | 0.002 | -0.17 |
| Leaf b* | -0.008 | -0.177 | **0.104** | -0.016 | -0.013 | 0.045 | 0.061 | 0.020 | -0.012 | 0.087 | 0.029 | 0.092 | -0.003 | 0.21 |
| LW | -0.006 | 0.046 | -0.022 | **0.078** | 0.002 | 0.518 | -0.574 | 0.012 | -0.498 | 0.495 | -0.109 | -0.188 | -0.005 | -0.25 |
| PL | 0.000 | -0.115 | 0.062 | -0.008 | **-0.022** | 0.293 | -0.098 | 0.034 | 0.129 | -0.131 | 0.288 | -0.159 | 0.009 | 0.28 |
| AFM | -0.013 | 0.007 | 0.004 | 0.034 | -0.005 | **1.205** | -0.789 | 0.025 | 0.498 | -0.517 | 0.195 | -0.131 | 0.008 | 0.52** |
| FN | 0.017 | -0.023 | 0.007 | -0.047 | 0.002 | -0.984 | **0.966** | -0.038 | -0.025 | 0.016 | -0.072 | 0.232 | -0.007 | 0.04 |
| Uniform | 0.006 | 0.034 | -0.024 | -0.011 | 0.009 | -0.352 | 0.424 | **-0.086** | 0.147 | -0.198 | -0.022 | 0.232 | -0.007 | 0.15 |
| TSS | 0.010 | -0.007 | 0.001 | 0.033 | 0.002 | -0.510 | 0.021 | 0.011 | **-1.177** | 1.236 | -0.220 | -0.175 | -0.016 | -0.79** |
| DMC | 0.009 | -0.016 | 0.007 | 0.031 | 0.002 | -0.500 | 0.012 | 0.014 | -1.168 | **1.246** | -0.238 | -0.174 | -0.015 | -0.79** |
| Fruit a* | 0.001 | 0.019 | -0.007 | 0.020 | 0.015 | -0.549 | 0.163 | -0.004 | -0.605 | 0.693 | **-0.428** | 0.069 | -0.009 | -0.62** |
| IBD | -0.005 | 0.036 | -0.025 | 0.039 | -0.009 | 0.412 | -0.587 | 0.052 | -0.538 | 0.566 | 0.077 | **-0.382** | 0.004 | -0.36 |
| PEN | 0.012 | -0.012 | 0.011 | 0.017 | 0.009 | -0.411 | 0.278 | -0.024 | -0.781 | 0.820 | -0.157 | 0.064 | **-0.023** | -0.20 |
| Residual | 0.023 | | | | | | | | | | | | | |

**P≤0.01, Yield $r_g$: Yield genotypic correlation; Direct and indirect effects contributing more than 20% of the partitioned genotypic correlation are highlited in green

**Table 5.14** Partitioning of genotypic correlations into direct (bold) and indirect effects of Chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) on yield in Oudtshoorn 2018/2019 (E5)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | FN | Uniform | TSS | DMC | Fruit a* | IBD | PEN | Yield $r_g$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | **2.912** | -1.644 | 0.063 | -0.132 | -1.270 | -2.036 | 1.694 | -0.185 | 2.764 | -6.521 | 3.588 | -0.073 | 0.707 | -0.13 |
| Leaf a* | 1.231 | **-3.889** | 0.068 | -0.064 | -0.279 | -0.783 | 0.410 | -0.003 | 0.500 | -1.785 | 2.150 | 0.667 | 1.460 | -0.32 |
| Leaf b* | -1.753 | 2.544 | **-0.105** | 1.518 | 1.037 | 0.533 | -0.295 | 0.132 | 0.706 | -0.366 | -2.837 | -0.785 | 0.054 | 0.38 * |
| LW | 0.184 | -0.119 | 0.076 | **-2.087** | -0.878 | 1.218 | -1.082 | -0.033 | 2.532 | -3.301 | 2.070 | 0.641 | 0.320 | -0.46 * |
| PL | -1.361 | 0.399 | -0.040 | 0.674 | **2.718** | -0.227 | 0.088 | 0.161 | 0.727 | -0.681 | -0.668 | -0.089 | -1.628 | 0.07 |
| AFM | -2.283 | 1.172 | -0.021 | -0.978 | -0.237 | **2.598** | -1.710 | 0.143 | -2.241 | 5.266 | -1.978 | 0.273 | 0.169 | 0.17 |
| FN | 2.543 | -0.822 | 0.016 | 1.165 | 0.123 | -2.290 | **1.940** | -0.192 | -1.153 | 0.105 | 0.573 | -0.486 | -1.311 | 0.21 |
| Uniform | 0.966 | -0.020 | 0.025 | -0.125 | -0.787 | -0.665 | 0.669 | **-0.557** | -3.781 | 7.124 | -0.477 | -0.204 | -1.872 | 0.30 |
| TSS | 0.674 | -0.163 | -0.006 | -0.443 | 0.165 | -0.488 | -0.187 | 0.176 | **11.939** | -21.165 | 3.311 | 0.356 | 5.223 | -0.61 ** |
| DMC | 0.896 | -0.328 | -0.002 | -0.325 | 0.087 | -0.645 | -0.010 | 0.187 | 11.924 | **-21.192** | 3.231 | 0.314 | 5.297 | -0.56 ** |
| Fruit a* | 2.157 | -1.726 | 0.061 | -0.892 | -0.374 | -1.060 | 0.229 | 0.055 | 8.161 | -14.134 | **4.844** | 0.299 | 1.769 | -0.61 ** |
| IBD | -0.161 | -1.967 | 0.062 | -1.014 | -0.182 | 0.538 | -0.715 | 0.086 | 3.224 | -5.048 | 1.098 | **1.319** | 2.479 | -0.28 |
| PEN | 0.305 | -0.841 | -0.001 | -0.099 | -0.655 | 0.065 | -0.377 | 0.154 | 9.240 | -16.634 | 1.270 | 0.484 | **6.749** | -0.34 |
| Residual | -0.768 | | | | | | | | | | | | | |

*P≤0.05, **P≤0.01, Yield $r_g$: Yield genotypic correlation; Direct and indirect effects contributing more than 20% of the partitioned genotypic correlation are highlited in green

**Table 5.15** Partitioning of genotypic correlations into direct (bold) and indirect effects of Chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform), total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) on yield in Oudtshoorn 2019/2020 (E6)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | FN | Uniform | TSS | DMC | Fruit a* | IBD | PEN | Yield $r_g$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| CHL | **-0.515** | -0.268 | 0.489 | 0.024 | -0.011 | -2.572 | 0.400 | 0.105 | -1.655 | 0.109 | 2.152 | 1.218 | 0.410 | -0.11 |
| Leaf a* | -0.175 | **-0.791** | 1.293 | -0.259 | -0.096 | -2.999 | 0.201 | 0.605 | 0.791 | -0.090 | 0.947 | 0.399 | -0.903 | -1.08 ** |
| Leaf b* | 0.165 | 0.670 | **-1.527** | 2.289 | 0.065 | 1.130 | 0.002 | -0.451 | -0.440 | 0.090 | -0.176 | -1.017 | 0.142 | 0.94 ** |
| LW | 0.003 | -0.047 | 0.802 | **-4.356** | -0.012 | 1.583 | -0.298 | 0.030 | -0.411 | -0.005 | -0.151 | 1.637 | 0.689 | -0.54 ** |
| PL | 0.049 | 0.644 | -0.841 | 0.429 | **0.119** | 1.776 | -0.190 | -0.220 | -1.908 | 0.154 | 0.326 | 0.119 | -0.224 | 0.23 |
| AFM | 0.403 | 0.722 | -0.524 | -2.096 | 0.064 | **3.288** | -0.418 | -0.289 | 0.384 | -0.008 | -2.077 | 0.308 | 0.597 | 0.35 |
| FN | -0.437 | -0.338 | -0.008 | 2.752 | -0.048 | -2.916 | **0.471** | 0.061 | -1.187 | 0.073 | 1.774 | 0.055 | -0.205 | 0.05 |
| Uniform | -0.055 | -0.486 | 0.698 | -0.134 | -0.026 | -0.966 | 0.029 | **0.985** | 0.468 | -0.055 | -0.887 | 0.332 | -0.568 | -0.66 ** |
| TSS | -0.138 | 0.101 | -0.109 | -0.290 | 0.037 | -0.204 | 0.091 | -0.075 | **-6.173** | 0.450 | 3.341 | 1.465 | 1.770 | 0.27 |
| DMC | -0.124 | 0.158 | -0.303 | 0.045 | 0.040 | -0.058 | 0.077 | -0.120 | -6.159 | **0.451** | 3.105 | 1.413 | 1.797 | 0.32 |
| Fruit a* | -0.222 | -0.150 | 0.054 | 0.132 | 0.008 | -1.366 | 0.167 | -0.175 | -4.128 | 0.280 | **4.997** | 0.169 | 0.053 | -0.18 |
| IBD | -0.218 | -0.110 | 0.539 | -2.475 | 0.005 | 0.351 | 0.009 | 0.114 | -3.140 | 0.221 | 0.292 | **2.881** | 1.837 | 0.31 |
| PEN | -0.081 | 0.273 | -0.083 | -1.147 | -0.010 | 0.750 | -0.037 | -0.214 | -4.174 | 0.310 | 0.102 | 2.022 | **2.617** | 0.33 |
| Residual | -0.706 | | | | | | | | | | | | | |

**P≤0.01, Yield $r_g$: Yield genotypic correlation; Direct and indirect effects contributing more than 20% of the partitioned genotypic correlation are highlited in green

In E6 (Table 5.15), characteristics with strong direct effects on yield included Fruit a* (5.00), AFM (3.29), IBD (2.88), PEN (2.62), LW (-4.36) and TSS (-6.17). Strong indirect effects of characteristics on yield were more evenly distributed but were mostly via LW, AFM, TSS and Fruit a*. The ranking of individual path coefficient analyses based on residual effects was E3 (0.02), E2 (0.04), E1 (-0.61), E6 (-0.71) and E5 (-0.77).

**5.5 Discussion**

Genetic improvement of both qualitative and quantitative characteristics of any crop depends on the amount of genetic variation available in the base population. To quantify this variation should be the first step in any improvement programme (Aruah *et al*., 2012). In the current study, phenotypic variance was partitioned into genotypic (heritable) and environmental (non-heritable) components for all characteristics studied, for both across environments and individual environments. The highly significant differences between genotypes for all characteristics implied the existence of inherent genetic variability and that all traits could be improved through selection. The greater the genetic variability, the greater the scope for improvement through selection (Naik and Prasad, 2016). The significant G x E interaction effects for all characteristics, except for Leaf b*, LW and PEN, suggested differential performance of hybrids in different environments and that selection efficiency could be influenced by environmental conditions.

Phenotypic and genotypic variances are indicators of the available variation for a characteristic, but it is impractical to compare different traits with one another since different units are used for different traits (Hamidou *et al*., 2018). By dividing variances with the population means, units are eliminated and PCV and GCV estimations are comparable for different traits. Larger coefficients of variance is an indication of higher levels of variation. In this study, the PCV was generally higher than the GCV for all characteristics, suggesting the influence of the environment. In the combined analysis across environments, the magnitude of the differences between PCV and GCV was higher than expected, but this can be attributed to the G x E interaction component. The AFM had the smallest margin expressed as a percentage of the PCV (data not shown), followed by FN. This was consistent with the analyses for the individual environments where the margin of difference between PVC and GCV was less than 10% of the PVC in six of the environments for AFM and less than 15% in five of the environments for FN. The DMY consistently showed high margins of difference between PCV and GCV for five of the environments, while that for all other characteristics varied substantially between the environments. This indicated the influence of the environment on the phenotypic expression of genotypes for various traits. A higher chance of improvement

of AFM and FN through selection, based on phenotypic performance, can therefore be expected as compared to the other characteristics (Manal, 2009).

Regardless of the difference between PCV and GCV, a higher GCV makes improvement through selection of specific characteristics more feasible (Kumar *et al*., 2013). Values of PCV and GCV greater than 20 were regarded as high, values between 10 and 20 as medium and values less than 10 were considered to be low (Deshmukh *et al*., 1986). Both AFM and FN demonstrated high PCV and GCV in the combined analysis across environments and this corresponded with results for all individual environments. In literature, PCV respectively for FN and AFM ranged from 14 (Naik and Prasad, 2016) to 62 (Gomes *et al.*, 2020) and 14 (Jahan *et al*., 2012) to 176 (Pandey *et al*., 2008). GCV ranged from 11 (Naik and Prasad, 2016) to 47 (Pandey *et al*., 2008) for FN and 11 (Jahan *et al*., 2012) to 56 (Gomes *et al.*, 2020) for AFM. In this study PCV (21.06) and GCV (11.1) for yield were lower than those values cited in literature, which ranged from 27 (Naik and Prasad, 2016) to 82 (Pandey *et al*., 2008) and 22 (Jahan *et al*., 2012) to 77 (Pandey *et al*., 2008) for PCV and GCV respectively. The variability for AFM and FN in this study seemed to represent the variability found in other reports. The yield variability might have been lower, since here the material was restricted to butternut types acceptable as commercial hybrids, whereas other studies included any type of *C. moschata* and included commercial hybrids, interspecies crosses and landraces.

A GCV value suggests the extent of genetic variability present within the material but does not provide a clear indication of the variation that is heritable. The ratio between GCV and PCV represents the $h_{bs}^2$ and denotes the proportion of genetically caused variation compared to phenotypic variation, where phenotypic variation includes both environmental and genetic variation (Naik and Prasad, 2016). The most important function of $h_{bs}^2$ lies in the predictive role it plays, in combination with the phenotypic standard deviation of the population. A higher level of $h_{bs}^2$ will result in a greater response to selection and will consequently advance a population in a desired direction (Acquaah, 2012). It also suggests the limited influence of the environment on the expression of the genotype in the phenotype. According to Singh (2001) (as cited in Rosmaina *et al*., 2016), $h_{bs}^2$ values greater than 0.80 are very high, values from 0.60 to 0.79 are moderately high, values from 0.40 to 0.59 are medium and values less than 0.40 were low. The $h_{bs}^2$ estimated in this study was moderately high for AFM, FN and PEN across locations. One would expect these characteristics to be easily improved through selection, but GAM varied greatly among these traits. The GAM was categorised as low (<10%), moderate (10% to 20%) and high (>20%) (Rosmaina *et al*., 2016). The yield component characteristics, AFM and FN demonstrated very high GAM with percentages

above 40%. Fruit quality characteristics including TSS, DMC, Fruit a* and PEN recorded GAM values around 20%, which suggested that significant improvement through selection may be possible. Leaf a*, LW and DMY had GAM lower than 10%, which may proof to be difficult to improve through selection using the current genotypes as base population.

When analysed across environments, a medium $h^2_{bs}$ for CHL resulted in a GAM of 10.5. However, in E2, $h^2_{bs}$ and GAM was 0.77 and 20.5%, respectively. This indicated that good progress could be made through selection under those specific environmental conditions, which are conducive to the expression of higher phenotypic variation. The opposite applies in those environments where phenotypic variation was suppressed by environmental conditions. The $h^2_{bs}$ estimate for Leaf a* across environments was very low, although E3 recorded a $h^2_{bs}$ of 0.85 with a corresponding GAM of -15.71%. The $h^2_{bs}$ for Leaf b* was more consistent, with an estimate of 0.57 across environments and the lowest estimation for the individual environments of 0.50 in E4. This resulted in a moderate GAM across environments, as well as for all individual environments. Due to limited variation and low PCV, LW consistently displayed low GAM. Across environments, PL displayed a $h^2_{bs}$ of 0.59 but under E3 and E4 conditions, $h^2_{bs}$ values close to 0.80 were recorded with a GAM close to 20%. Although the GCV for all plant characteristics was low across environments, improvement through selection is still possible, but in specific environments only. However, since these characteristics are of low monetary value, it would be difficult to motivate for a breeding programme in these specific environments for improvement of any of these traits without evidence of a strong association with higher monetary value traits. For selection to be more effective, genetic variation for these characteristics in the base population would have to be increased.

Across all six environments, both AFM and FN had high $h^2_{bs}$, coupled with a very high GAM, suggesting a possible increase of 55% in fruit mass and a 44% increase in fruit number, with a 5% selection intensity after one selection cycle. This pattern was also observed in data for all of the individual environments. This is supported by examples in literature, where $h^2_{bs}$ for FN was estimated as 0.65 (Kumar *et al*., 2011), 0.58 (Naik and Prasad, 2016) and 0.70 (Pandey *et al*., 2008). Kumar *et al*. (2011) estimated GAM at 79% but worked with a higher GCV, while Pandey *et al*. (2008) recorded GAM at 17%, but where GCV was lower. Various other studies also reported low $h^2_{bs}$, yet they were still able to estimate GAM of 14% (Akter *et al*., 2013) and 22% (Jahan *et al*., 2012). The same applies to AFM, where high $h^2_{bs}$ estimates ranged from 0.61 (Jahan *et al*., 2012) to 0.97 (Mohsin *et al*., 2017), with GAM values of 57% (Kumar *et al*., 2011) and 66% (Sultana *et al*., 2015). Reports of AFM $h^2_{bs}$ values below 0.40 still managed to estimate GAM values of 21% (Akter *et al*., 2013) and 17% (Naik and Prasad,

2016). Although an improvement of AFM should be easy, fruit sizes are related to specific market segments and should, therefore, not necessarily be increased. An increase in FN will be beneficial but only when accompanied by no significant change in AFM. In this study, DMY $h^2_{bs}$ fluctuated a great deal between environments, resulting in respectively a low $h^2_{bs}$ and GAM for the combined analysis across environments.

For E1, the $MS_e$ was larger than the $MS_g$, resulting in a negative genotypic variance and as a result, $h^2_{bs}$ and GAM could not be calculated. Estimates of $h^2_{bs}$ and GAM for the remaining environments ranged from 0.24 to 0.83 and from 6.7% to 48.5% respectively, suggesting that success in improving DMY will be greatly influenced by the environment. Estimates of $h^2_{bs}$ and GAM for yield also fluctuated significantly among environments. Environments E1 and E6 had very high residual MS values for yield, which were attributed to trial management practices. Both these environments had very low yield $h^2_{bs}$ estimates, resulting in a low estimation for the combined analysis across environments. Estimates of $h^2_{bs}$ for the remaining four environments were all above 0.65. Literature refers to yield $h^2_{bs}$ ranging from 0.50 (Jahan *et al*., 2012; Gomes *et al.*, 2020) to 0.95 (Kumar *et al*., 2011; Nagar *et al*., 2017), while GAM ranged from 40% (Naik and Prasad, 2016; Nagar *et al*., 2017) to more than 100% (Sultana *et al*., 2015). Those studies were conducted in a single location and a single season and are, therefore, more in agreement with our individual environment estimations. Estimates of $h^2_{bs}$ for uniformity were low for E3 and E4, medium for E1 and E2, and moderately high for E5 and E6, with the latter demonstrating high GAM. The $h^2_{bs}$ estimates for the combined analysis across environments were low; suggesting that improvement through simple selection for uniformity may not result in the desired outcome and should be limited to E5 and E6. Improvement through selection for yield and yield component characteristics can be very effective, although selection for certain traits should be limited to specific environments.

Medium $h^2_{bs}$, coupled with moderately high GAM, were estimated for both TSS and DMC for the combined analysis across environments as well as for the individual locations. An increase of nearly 20% in both TSS and DMC is possible after a single round of selection. Estimates of $h^2_{bs}$ for DMC and TSS have previously been recorded as 0.98 and 0.26 (Akter *et al*., 2013) and GAM as 43% and 6%, respectively. Mohsin *et al*. (2017) also reported TSS $h^2_{bs}$ of 0.98. However, both GCV and PCV was higher for DMC and lower for TSS as compared to this study.

Estimates of $h^2_{bs}$ and GAM for Fruit a* fluctuated, ranging from 0.57 to 0.78 and from 17.7% to 38.4% respectively, although the combined analysis across environments yielded a $h^2_{bs}$ and

GAM of 0.56 and 24.5%, respectively. Although the environment played a significant role in Fruit a*, significant improvements is attainable in most of the environments. PEN produced moderately high to very high $h_{bs}^2$ estimates for the individual environments, with GAM ranging from 17.0% to 23.2%, suggesting moderate to high progress in various environments. For IBD, $MS_e$ was equal to $MS_g$ in E4 and, as a result, $\sigma_g^2$, $h_{bs}^2$ and GAM could not be estimated. For the remaining environments, with the exception of E3, $h_{bs}^2$ ranged from 0.44 to 0.75 with high estimates for GAM. Meaningful improvement of internal fruit quality through selection would be possible across all environments.

Estimations of $h_{bs}^2$ are reliable if accompanied by high GA estimates. Characteristics that demonstrated both high $h_{bs}^2$ and at least moderately high GAM for the combined analysis across environments were AFM, FN and PEN. These traits are most probably under additive genetic control, where simple phenotypic selection in the early generations could be expected to have a positive outcome (Muhder *et al*., 2020). The characteristics of CHL, Leaf b*, LW, PL and IBD have medium $h_{bs}^2$ and lower GAM and although these are still under additive genetic control, inheritance is more complicated. Environmental effects, as well as non-additive genetic control play a larger role in contributing to phenotypic variation. The characteristics Leaf a*, DMY, uniformity and yield demonstrated relatively low $h_{bs}^2$, with significant influence from the environment that would result in slow progress. For these characteristics, simple selection may not produce the desired results for improvement (Manal, 2009; AL-Tabbal and Al-Fraihat, 2012). As mentioned before, no characteristic can be bred in isolation and, therefore, associations between characteristics and yield can assist in finding a more effective approach.

For most of the characteristics, Bartlett's test (1937) confirmed that the variances among environments were not homogeneous. Therefore, it would be inappropriate to combine data into a single correlation analysis across environments. A set of correlation analyses for each individual environment indicated significant associations between a number of characteristics, both on phenotypic and genotypic level. In most cases, the genotypic correlation coefficients, which indicated the heritable association between two variables (Abebe *et al*., 2020), were higher than the corresponding phenotypic correlation coefficients, in a similar direction. This indicated that the environment suppressed correlations at phenotypic level. This, in turn, corresponded with the observation that the strength and significance of correlations fluctuated among environments, and were highly influenced by environmental conditions. This was in accordance with a study on *Cucurbita* spp. in Nigeria (Aruah *et al*., 2012).

According to Itle and Kabelka (2009), correlation coefficients could be described as strong (>0.85), moderate (between 0.84 and 0.75) and weak (<0.74). However, it should be mentioned that sizes of correlations are directly related to the size of the dataset. The larger the dataset, the lower the correlations. The most relevant factor is the significance of the correlation values, where very low correlations can be significant in a very large dataset. At genotypic level, significant and moderate correlations were observed between CHL and yield component characteristics. Although the associations were significant on phenotypic level, they were much weaker and this indicated strong environmental influence. The leaf characteristics also had moderately positive and moderately negative associations with AFM and FN respectively, suggesting that a higher CHL level is associated with not only smaller fruit, but also with more fruit per plant. These associations with AFM occurred in four of the individual environments and with FN in five different environments. The CHL did not have a significant association with yield itself, similarly to what was recorded in maize where a correlation of 0.31 between CHL and grain yield was estimated (Ghimire *et al.,* 2015). The contrary was observed in dry bean where CHL had a non-significant correlation with seed yield (Guler and Ozcelik, 2007).

A strong negative association was observed between Leaf a* and Leaf b* at both phenotypic and genotypic level. This suggested that as canopy colour approach the green spectrum in the red-green range, canopy colour simultaneously approaches blue in the yellow-blue spectrum. With the exception of CHL, none of the plant characteristics demonstrated meaningful associations with any of the economically important traits, on either genotypic or phenotypic level. Thus, the plant characteristics will be of limited use for indirect selection with the aim of improvement of traits of monetary value. In a separate study, which included 41 *Cucurbita* spp. inbred lines, similar results were found where no significant correlations were observed between any economical important traits and leaf colour (Du *et al*., 2011). The same study reported LW to have a significant phenotypic correlation of 0.59 with AFM and this observation was in agreement with the current study where genotypic correlations of 0.54 (E1) and 0.60 (E4) were recorded.

A very strong negative genotypic association was observed between AFM and FN, suggesting that FN increases when AFM decreases. This association was present in all individual environment analyses and, although influenced by the environment, phenotypic correlations across all environments were also significant and higher than -0.69. Numerous studies have reported this correlation to be non-significant (Pandey *et al*., 2008; Akter *et al*., 2013; Grisales *et al*., 2015; Mohsin *et al*., 2017) but Tamilselvi *et al*. (2012) reported a significant genotypic correlation of -0.57 between AFM and FN. Various significant correlations have been recorded

in literature, which could not be supported with the current study. Some of these included correlations between FN and AFM with yield of 0.99 and 0.58 respectively (Akter *et al*., 2013) and correlations between yield and AFM of higher than 0.80 (Grisales *et al*., 2015; Mohsin *et al*., 2017). Correlations between yield and AFM as well as yield and FN smaller than 0.5 were, however, reported (Pandey *et al*., 2008; Naik *et al*., 2015; Sultana *et al*., 2015), which confirmed results presented here. Collectively it seems that yield cannot be reliably improved through indirect selection of either AFM or FN.

Weak negative genotypic correlations existed between yield and internal fruit quality characteristics, which included TSS, DMC and Fruit a*. Since both yield and production of complex biochemical compounds, such as carbohydrates and carotenoids, are dependent on the photosynthetic potential of the plant, these negative associations were expected. Since both yield and fruit quality traits are influenced by the environment, these correlations were only evident on a genotypic level and in some of the environments. These correlations imply that the fruit mesocarp sugar and starch contents would decrease as yield increases. A correlation of -0.63 between yield and TSS was reported by Akter *et al*. (2013), which supports the current study. However, Mohsin *et al*. (2017) reported the opposite, with a correlation of 0.38. Since Fruit a* is an indication of the red colour spectrum observed, which is linked to carotenoid content, an increase in yield would be accompanied by a reduction of the red colour pigment in fruit flesh.

The strong significant positive correlations, both at phenotypic and genotypic level between TSS and DMC across all environments, agreed with a similar study in which a genotypic correlation of 0.98 between TSS and DMC was reported (Hernandes *et al*., 2020). Although genotypic correlations were slightly higher than phenotypic correlations, these were by a small margin and indicated less environmental influence. Both TSS and DMC demonstrated weak positive genotypic correlations with PEN across all environments, while DMC showed weak positive genotypic correlations with Fruit a* in three of the environments. Since these correlations do not occur in all environments and phenotypic correlations are much weaker, any changes in the environment have a significant impact on the presence or absence of these correlations.

Overall, inconsistencies in results across different environments could be attributed to the differences among the environments. This would also explain contradictory results reported in literature. Since genetic parameters are dependent on a specific population and a specific environment in which they are measured, it is expected that estmations of genetic parameters from studies using different genotypes cultivated under different conditions, would not necessarily be the same.

Yield is arguably the most important characteristic in any breeding programme and is influenced not only by the environment but has also a complex inheritance. Yield is associated with various morpho-agronomic and internal fruit characteristics, and these can be interrelated. These underlying relationships often affect the direct relationship with yield, rendering correlation coefficients unreliable for use as selection indices (Aruah *et al*., 2012). Thus, a path coefficient analysis was performed in order to partition all genotypic correlations and establish the direct and indirect effects of each characteristic on yield. Results from the separate analyses accentuated differences among the environments and this was supported by the differences among the residual effects. The residual effects estimated for E1, E5 and E6 were larger than 0.5, indicating that while the traits investigated in this study do influence yield, they are not the main determinants of fruit yield in butternut. This is not unusual, since there are other reports on *C. moschata* where similar residual effects were reported (Pandey *et al*., 2008; Aruah *et al*., 2012). However, the path coefficient analysis of E2 and E3 resulted in residual effects smaller than 0.1 and, therefore, captured most of the direct and indirect effects on yield.

With the exception of PL, all plant characteristics had strong negative direct impact on yield in E1. However, all of them were countered by strong positive indirect effects, which resulted in mostly meaningless correlations with yield. Although the direct effect of CHL (-1.83) on yield was softened by the sum of the indirect effects, the result was still in a weak but meaningful significant genotypic correlation (-0.71). While FN (1.86) had a strong positive direct effect on yield, it had negative indirect effects via CHL (-1.32), Leaf a* (-2.19) and IBD (-0.87), resulting in a significant negative correlation (-0.68). The sum of the indirect effects masked the strong direct effect of uniformity (1.26), resulting ultimately in a non-significant correlation. The opposite occurred for TSS (-0.01) where a weak direct effect was enhanced via indirect effects, resulting in a strong significant correlation with yield. Direct effects of Fruit a* (-0.97) and PEN (-0.93) were slightly suppressed by the indirect effects but remained as significant negative correlations. Yield is strongly and directly affected by IBD (2.13) but was completely nullified by indirect effects. In E1, various characteristics demonstrated meaningful indirect effects on yield, mostly via Leaf a* and Leaf b*.

Meaningful genotypic correlations in E2 were between yield and LW (-0.70), yield and Leaf b* (0.43), yield ad FN (0.58), and could be attributed mostly to the direct effect of these characteristics on yield. Although a strong negative indirect effect on yield via IBD was observed, it was countered by a number of indirect effects, which culminated in a negligent total sum of indirect effects. Various meaningful direct effects on yield were estimated but

these were countered by the indirect effects of other characteristics. The direct positive impact of FN (0.62) on yield was countered by negative indirect effects from AFM (-0.42) and DMC (0.43), resulting in a correlation lower than 0.6. The strong direct effect of DMC (-2.15) was almost nullified by the indirect effects of all the remaining fruit quality characteristics. Fruit a* (0.90) and IBD (0.92) had meaningful positive direct effects on yield, which were completely reversed by a strong negative indirect effect of DMC. Based on results from E2, eight characteristics had strong indirect effects on yield through DMC; therefore, DMC should be included as part of an evaluation process for the improvement of yield in butternut.

Strong direct effects of AFM (1.21) and FN (0.97) in E3 were both weakened by indirect effects of the alternative characteristic, resulting in meaningless genotypic correlations with yield. For fruit quality traits, TSS (-1.18) had a strong direct effect on yield, which was softened mostly by the indirect effect of DMC, yet it remained a significant negative correlation. The direct positive effect of DMC (1.25) on yield was completely reversed by the sum of the indirect effects dominated by TSS (-1.17) and which resulted in a significant negative correlation. The indirect effect of AFM (-0.55), TSS (-0.61), DMC (0.69), together with the direct effect of Fruit a* (-0.43), all contributed to a weak negative significant correlation between yield and Fruit a*. The indirect positive effects of AFM, FN, TSS and DMC were the most important influences of the correlation estimates for yield.

Strong direct effects were estimated for CHL (2.91), Leaf a* (-3.89), LW (-2.09), PL (2.72), AFM (2.60) and PEN (6.75) in E5. However, these were all reduced to meaningless correlations with yield by indirect effects of various characteristics of which TSS and DMC were the most influential. Strong positive direct effects of TSS (11.94) and Fruit a* (4.84) were reduced to significantly negative correlations, mostly through the indirect effects dominated by DMC. A strong negative direct effect of DMC (-21.19) was countered by indirect effects of TSS, Fruit a* and PEN, but a significant negative correlation remained with yield. The characteristics that exerted the most influential impact, indirectly on yield, were TSS and DMC.

A genetic correlation of -1.08 between yield and Leaf a* was over estimated in E6 and can be explained by the large residual MS estimated for yield (data not shown), which would have impacted the accuracy of all the calculations for this environment. The correlation was due to the direct effect of Leaf a* (-0.79) as well as indirect effects dominated by Leaf b* and AFM. A strong direct effect was recorded for Leaf b* (-1.53), which was reversed to a positive correlation mostly through an indirect effect of LW. Additional strong direct effects included LW (-4.56), AFM (3.29), TSS (-6.17), Fruit a* (5.00), IBD (2.88) and PEN (2.62). All of these direct effects were, however, nullified by a set of indirect effects dominated by LW, AFM, TSS

and Fruit a*. Uniformity (0.99) had a strong direct effect, but this too was reversed to a significant negative correlation by indirect effects dominated by AFM and Fruit a*. In E6, the characteristics of LW, AFM, TSS and Fruit a* all contributed a number of indirect effects on yield.

Overall, yield is clearly influenced by a number of both direct and indirect effects, but only FN had a recurring positive direct effect across all environments. None of the characteristics had a consistently negative direct influence on yield. In four of those environments, AFM and IBD had positive direct effects on yield, with LW having a negative effect in four environments. The negative direct IBD effect and positive direct LW effect were both estimated for E3. The characteristics CHL, uniformity and Fruit a* consistently had a positive indirect impact on yield via FN across all environments, which confirmed the importance of FN. Yield is clearly a complex characteristic that was influenced in some or other way by all of the characteristics that were evaluated in this study. Although a strong significant genotypic correlation between FN and yield was absent, it would nonetheless be beneficial to be included in any yield improvement programme.

There are a number of path coefficient analyses for *C. moschata* where characteristics describing earliness of, and position of male and female flowers (Lawal, 2009; Akter *et al*., 2013; Sultana *et al*., 2015; Mohsin *et al*., 2017), as well as plant architecture and fruit dimensions (Pandey *et al*., 2008), were included in combination with yield components. Of those studies, Pandey *et al*. (2008) reported AFM as having a strong negative direct effect on yield, which was in contrast to the data presented here. The remaining authors all reported AFM and FN as having positive direct effects on yield, as seen here in environments E2, E3, E5 and E6. Additional studies estimated DMC (Lawal, 2009) and TSS (Mohsin *et al*., 2017) as having negligible direct effects on yield, which corresponds with E1 only. Sultana *et al*. (2015) claimed a strong negative direct effect of LW on yield, which was seen for environments E1, E2, E5 and E6. In a study repeated over two seasons, individual path coefficient analyses indicated FN had a strong positive direct effect on yield in the first season, but was followed by a strong negative direct effect on yield in the second (Aruah *et al*., 2012). Path coefficient analysis is dependent on genotypes and environments, and contradicting results across different environments within a study should, therefore, be expected. This explains why results here did not always correspond with those cited in literature.

In grain yield, selection based on yield alone is usually not as effective when compared to selection together with its component characteristics (Fasahat *et al*., 2016). This is also reflected in studies on crops such as boro rice (Chakraborty *et al*., 2010), cowpea (Fasahat *et*

*al*., 2016) and even *C. moschata* (Ahmed *et al*., 2017). Data generated in this study also supported the assumption that yield is a complex inherited characteristic and should not be considered as a stand-alone characteristic. The best approach for the improvement of yield would be to simultaneously consider not only yield, but also FN, AFM and to a lesser extent IBD and LW as having direct effects. The indirect effects of DMC and TSS should also be considered. Since DMC and TSS are negatively associated with yield, parallel breeding programmes for the improvement of internal fruit quality and various yield component characteristics should be considered independently. Improved traits could then be combined through hybridisation in economically viable $F_1$ crosses.

### 5.6 Conclusions

High levels of both genotypic and phenotypic variance within all characteristics studied indicated the presence of inherent variability that can be utilised for the improvement of butternut. The estimation of PCV indicated high levels of variation within plant and internal fruit characteristics and very high levels for yield and yield component characteristics. The GCV for plant characteristics was lower when compared to that of other traits in this study, suggesting that the improvement of plant characteristics through selection would be more challenging. A narrow range of difference between PCV and GCV for AFM and FN indicated that these traits are governed predominantly by genetic factors, with limited environmental influence on the phenotype. Selection based on phenotypic values could, therefore, be effective. The characteristics AFM, FN, TSS, DMC, Fruit a* and PEN had high $h_{bs}^2$ estimates, accompanied by high GAM and, as a result, selection in early generations would be effective for improvement of these traits. Low $h_{bs}^2$ and low GAM estimates for Leaf a*, DMY, uniformity and yield suggested that progress through simple selection would be ineffective. Both genotypic and phenotypic correlations suggested that CHL has moderate associations with AFM and FN, with a strong negative genotypic correlation between the latter two traits. Yield also had a weak negative genotypic association with TSS, DMC and Fruit a*. A number of internal quality characteristics shared weak positive genotypic associations, of which the strongest was between DMC and TSS. Path coefficient analyses indicated FN and to a lesser extent AFM, IBD and LW, to have recurring direct effects on yield, and these should be considered in any programme for the improvement of yield in butternut.

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# CHAPTER 6

# COMBINING ABILITY, GENE ACTION AND HETEROSIS FOR MORPHO-AGRONOMIC AND INTERNAL QUALITY CHARACTERISTICS IN BUTTERNUT

## 6.1 Abstract

Both yield and internal fruit quality are central for the development and release of improved butternut hybrids, but the gene action involved with regard to the dependant characteristics is still not clear. Therefore, the aim of this study was to investigate the magnitude of general and specific combining ability variances to gain insight into the gene action involved in the control of the 15 morpho-agronomic and internal fruit quality characteristics studied. The heritability and heterosis of these characteristics were also investigated. A line x tester analysis involving 16 test crosses, generated by crossing four elite parental lines with four testers, was conducted on three locations over two seasons. None of the parents was found to be consistently good general combiners for all the traits. The line x tester interaction mean square values for leaf colour, fruit number, total soluble solids, dry matter content and fruit mesocarp density were not significant. For leaf width, petiole length, average fruit mass, green-red colour contribution in the fruit mesocarp and internal breakdown, the predictability ratios ($\sigma^2_{GCA}/\sigma^2_{SCA}$) were larger than one, which suggested that those traits were predominantly under additive genetic control. The general combining ability variance for chlorophyll content, dry matter yield, uniformity and yield were lower than the specific combining ability variance, suggesting that non-additive gene action was dominant. This was supported by a large difference between broad-sense and narrow-sense heritability estimates for these traits. The results also suggested the involvement of significant heterosis in all traits measured. The implication is that all characteristics measured can be improved through selection in early generations, although for yield, uniformity and dry matter yield, more emphasis should be placed on the exploitation of heterosis.

***Keywords*****:** *Cucurbita moschata*, combining ability variance, line x tester analysis, narrow-sense heritability

## 6.2 Introduction

*Cucurbita moschata* is a cross-pollinating, monoecious crop, where a large seed number per pollination event results in low seed production costs. The crop also has a low seed requirement per unit area, allowing exploitation of heterosis as an improvement strategy (Dubey *et al.,* 2014). The extensive phenotypic variability for most of the economically important characteristics and the limited number of commercial breeding programmes make

butternut squash breeding even more attractive. The development of suitable hybrids with high yields, good internal quality and disease resistance would be a desirable end-goal. A comprehensive understanding of the genetic behaviour of these desirable characteristics is crucial when formulating a suitable and efficient breeding programme (Pandey *et al.*, 2010). This can be done through line x tester analysis in which general combining ability (GCA) and specific combining ability (SCA) effects and their variances are measured. These variances can be used to determine whether a characteristic is under additive or non-additive genetic control, which will in turn determine whether a recurrent selection or heterosis breeding approach is used. In any breeding programme, the main objectives are to identify the best genotypes for future breeding in order to generate maximum variation in subsequent segregating populations, and to predict parental line combinations with a high proportion of desirable characteristics combined in one genotype for commercial release (Fasahat et al., 2016).

Many *Cucurbita* breeding studies have focussed on analyses of combining ability in order to identify ideal parents, and to recognise the mode of gene action of various characteristics. With the exception of one line x tester analysis (Tamilselvi *et al*., 2015), all studies involved diallel analysis and demonstrated both GCA and SCA variances to be significant for most, if not all, characteristics studied (Ahmed *et al.,* 2017; Kakamari and Jagadeesha, 2017; Mohsin *et al*., 2017; Restrepo-Salazar *et al.*, 2019). These suggested the involvement of both additive and non-additive gene action. There was general agreement among the studies that fruit weight, fruit per plant and yield were mostly under additive genetic control, with limited application for heterosis breeding (El-Tahawey *et al.*, 2015; Hussein and Hamed, 2015; Mohsin *et al*., 2017; Marxmathi *et al*., 2018). Two studies (Tamilselvi *et al.,* 2015; Kakamari and Jagadeesha, 2017), however, found these traits to be mostly under non-additive gene action and they suggested improvement through hybridisation.

Heterosis is the expression of superiority of a $F_1$ hybrid over the parents with regard to a specific characteristic, and is the deviation of the $F_1$ hybrid character value from the average value of both parents (Abrham *et al*., 2017). Heterosis has been widely used in agriculture for the improvement of yield and adaptability in various crops (Shafiin *et al*., 2020). However, due to limited inbreeding depression in *Cucurbita*, the expected levels of heterosis is much lower when compared to other cross-pollinating crops prone to extreme inbreeding depression such as *Zea maize* (Cardoso, 2004; Loy, 2004; Acquaah, 2012). Nevertheless, both positive and negative heterosis for different quantitative characteristics have been cited in various *Cucurbita* studies with mid-parent heterosis (MPH) where they reported heterosis as high as 171% for yield (Ahmed *et al*., 2017), 113% for fruit number (Jha *et al*., 2009) and 203% for

average fruit weight (Darrudi *et al*., 2018). These studies also included plant characteristics *viz*. vine length, days to first male and female flowers, first male and female node as well as fruit dimensions, but these are of limited economic importance. In contrast, genetic parameters of important fruit quality characteristics have received little attention.

To date commercial growers and breeders have focused mostly on yield and yield component characteristics, but in order to stay competitive in this market, seed companies need to place more emphasis on internal fruit quality. The aim of this study was to investigate the magnitude of GCA and SCA variances to gain insight into the gene action involved in the control of 15 morpho-agronomic and internal fruit quality characteristics. The objectives were to: (i) estimate combining ability effects; (ii) estimate additive gene action compared to non-additive gene action; and (iii) establish the magnitude of heterosis and heritability of morpho-agronomic and internal fruit quality characteristics in butternut. These findings will be incorporated into designing an appropriate breeding approach.

## 6.3 Materials and methods

### 6.3.1 Plant material, site description and trial design

Genotypes within a heterotic group express similar responses with regard to combining ability and heterosis when crossed with a genotype from a genetically removed group (Fasahat *et al.*, 2016). Therefore, four testers and four lines were selected from three different heterotic groups, which were constructed based on genotypic maker data, and covered phenotypic variation across the Starke Ayres butternut genepool. Based on historical data, the testers included parental lines with acceptable *per se* performance as well as acceptable performance in commercially available hybrids produced in combination with other parental lines. The lines had not previously been used in hybrid combinations, but had acceptable *per se* performance and displayed wide diversity for yield, yield components and internal fruit quality characteristics. A line x tester mating design was used to generate 16 hybrids. The reciprocals were not included. A total of 24 genotypes (including the 16 hybrids, four testers and four lines) were evaluated during the summers of 2018/2019 and 2019/2020 in three locations across South Africa: *viz*. Jacobsdal, Kaalfontein and Oudtshoorn. Refer to Chapter 3, Sections 3.3.1 to 3.3.2 for detailed descriptions regarding the sites, trial design and management, and plant material used in this study. Entries G1 to G4 were used as testers and G5 to G8 as lines, respectively in the statistical analysis.

### 6.3.2 Data collection and statistical analysis

Refer to Chapter 3, Section 3.3.3 for detailed descriptions regarding the collection of data on morpho-agronomic and internal fruit quality characteristics used in this study. Significant

interaction effects for season were identified for most of the studied characteristics as discussed in Chapter 3, and so these data were analysed as six individual environments.

Using a programme compiled by Genet (2016), the data from the line x tester design were subjected to several analyses of variance (ANOVA) using the General Linear Models Procedure (PROC GLM) of SAS software, version 9.4 (SAS Institute Inc., 2013).

A fixed-effects model was assumed for the genotypes (lines, testers and hybrids) and environments in the current study. The underlying statistical model for a line x tester analysis was assumed in the combining ability analysis (Arunachalam, 1974; Dabholkar, 1999):

$$y_{ijk} = \mu + f_i + m_j + mf_{ij} + r_k + e_{ijk}$$

Where, $y_{ijk}$ is the observation recorded on the $i \times j$ cross in the $k^{th}$ replication, μ is the population mean effect, $f_i$ is the GCA effect of the $i^{th}$ tester (female), $m_j$ is the GCA effect of the $j^{th}$ line (male), $mf_{ij}$ is the SCA effect of the $i \times j$ cross, $r_k$ is the $k^{th}$ replication effect and $e_{ijk}$ is the residual effect.

Combining ability can be defined as an estimation of the impact of a genotype, based on the performance of its progeny. The average performance of a genotype in a number of hybrid combinations is referred to as the GCA. SCA is the deviation of the actual performance of a hybrid, from the expected performance, based on the average performance of the parental lines involved (Fasahat *et al*., 2016). The GCA effect of the parents and the SCA effects of the hybrid crosses were estimated using the equations of Singh and Chaudhary (1985) and Tamilselvi *et al*. (2015):

$$\mu = \frac{x_{...}}{rlt}$$

GCA effects of the lines ($g_j$):

$$g_i = \frac{x_{i...}}{rt} - \frac{x_{...}}{rtl}$$

GCA effects of the testers ($g_j$):

$$g_j = \frac{x_{.j.}}{rl} - \frac{x_{...}}{rtl}$$

SCA effects of the hybrids ($s_{ij}$):

$$s_{ij} = \frac{x_{ij.}}{r} - \frac{x_{i..}}{rt} - \frac{x_{.j.}}{rl} + \frac{x_{...}}{rlt}$$

Where, $x_{...}$ is the total of the hybrids over $r$ number of replications, $x_{i...}$ is the total of the $i^{th}$ line over all the testers (female parents) and $r$ replications, $x_{.j.}$ is the total of the $j^{th}$ tester over all the lines (male parents) and $r$ replications and $x_{ij.}$ is the total of the hybrids between the $i^{th}$ line and $j^{th}$ tester over $r$ replications.

The GCA effect standard errors for the lines ($SE_l$) and testers ($SE_t$) and SCA standard error for the crosses ($SE_c$) were estimated from the equations below where $M_e$ is the mean square error. The determination of significance for the GCA ($t_{GCA}$) and SCA ($t_{SCA}$) effects were done with the relevant t-tests (Mbuvi *et al.*, 2018):

$$SE_l = \sqrt{(M_e/r \times t)}$$

$$SE_t = \sqrt{(M_e/r \times l)}$$

$$SE_c = \sqrt{(M_e/r)}$$

$t_{GCA} = g_i/SE_l$ or $t_{GCA} = g_j/SE_t$

$$t_{SCA} = s_{ij}/SE_c$$

GCA and SCA variances were derived from the ANOVA of the different traits using the equations of Singh and Chaudhary (1985) and Fellahi *et al*. (2013).

Covariance of half-sib of line:

$$Cov.H.S.(line) = \frac{M_l - M_{l\times t}}{rt}$$

Covariance of half-sib of tester:

$$Cov.H.S(tester) = \frac{M_t - M_{l\times t}}{rl}$$

Covariance of full-sib:

$$Cov.F.S = \frac{(M_l - M_e) + (M_t - M_e) + (M_{l\times t} - M_e)}{3r} + \frac{6rCov.H.S(avg) - r(l+t)Cov.H.S(avg)}{3r}$$

Covariance of half-sib (average):

$$Cov.H.S.(avg) = \frac{1}{r(2lt - l - t)}\left[\frac{(l-1)(M_l) + (t-1)(M_t)}{l + t - 2} - M_{l\times t}\right]$$

Where, $M_l$ is the mean square of the lines, $M_t$ is the mean square of the testers, $M_{l\times t}$ is the mean square of the lines x testers, $M_e$ is the mean square of the error, and $r$, $l$, and $t$ are the number of replications, lines and testers.

Assuming no epistasis, variance due to GCA ($\sigma^2_{gca}$) and variance due to SCA ($\sigma^2_{sca}$) were calculated as follows:

$$\sigma^2_{gca} = Cov.H.S.(avg) = \left(\frac{1+F}{4}\right)\sigma^2_A$$

$$\sigma^2_{sca} = \frac{M_{l\times t} - M_e}{r}$$

$$\sigma^2_{sca} = \left(\frac{1+F}{2}\right)\sigma^2_D$$

Additive and dominance genetic variances ($\sigma^2_A$ and $\sigma^2_D$) were calculated using an inbreeding coefficient equal to one ($F = 1$), because both lines and testers were inbred. The predictability ratio ($\sigma^2_{gca}/\sigma^2_{sca}$) and degree of dominance $(\sigma^2_D/\sigma^2_A)^{1/2}$ were used to rate the relative weight of additive versus non-additive type of gene actions (Fellahi *et al*., 2013).

Broad-sense heritability ($h^2_{bs}$) and narrow-sense heritability ($h^2_{ns}$) were calculated using the equations of Falconer and Mackay (1996) and Agaba *et al.* (2017):

$$h^2_{bs} = \frac{\sigma^2_{GCA(Female)} + \sigma^2_{GCA(Male)} + \sigma^2_{SCA}}{\sigma^2_{GCA(Female)} + \sigma^2_{GCA(Male)} + \sigma^2_{SCA} + \sigma^2_e}$$

$$h^2_{ns} = \frac{\sigma^2_{GCA(Female)} + \sigma^2_{GCA(Male)}}{\sigma^2_{GCA(Female)} + \sigma^2_{GCA(Male)} + \sigma^2_{SCA} + \sigma^2_e}$$

Where:

$$Phenotypic\ effects = \sigma^2_{GCA(Female)} + \sigma^2_{GCA(Male)} + \sigma^2_{SCA} + \sigma^2_e$$

$$Total\ genetic\ effects = \sigma^2_{GCA(Female)} + \sigma^2_{GCA(Male)} + \sigma^2_{SCA}$$

$$Additive\ gene\ effects = \sigma^2_{GCA(Female)} + \sigma^2_{GCA(Male)}$$

Where, $\sigma^2_{GCA(Female)}$ and $\sigma^2_{GCA(Male)}$ are the GCA variance of the female and male, $\sigma^2_{SCA}$ is the SCA variance and $\sigma^2_e$ is the error variance.

The proportional contribution of lines, testers and their interactions to total variance were calculated according to the equations of Singh and Chaudhary (1985):

$$Contribution\ of\ lines = \frac{SS(l) \times 100}{SS(Crosses)}$$

$$Contribution\ of\ testers = \frac{SS(t) \times 100}{SS(Crosses)}$$

$$Contribution\ of\ (l \times t) = \frac{SS(l \times t) \times 100}{SS(Crosses)}$$

Where, $SS$ refers to the sum of squares of the lines $(l)$, testers $(t)$, line x tester interactions $(l \times t)$ and crosses.

Mid-parent heterosis (MPH) was computed for each characteristic and significance was tested using a t-test (Abrham *et al*., 2017):

$$MPH = \frac{F_1 - MP}{MP} \times 100$$

$$t_{MPH} = \frac{F_1 - MP}{\sqrt{3M_e/2r}}$$

Where, $F_1$ refers to the mean of the $F_1$ hybrid, $MP$ is the mean of the mid-parent value (i.e. $[P_1 + P_2]/2)$, $M_e$ is the error mean square from the ANOVA table and $r$ is the number of replications. “Mid-parent heterosis” will hereafter be referred to as “heterosis” only.

### 6.4 Results

The combined ANOVA for the 24 genotypes evaluated across the six environments revealed highly significant (P≤0.001) differences amongst the genotypes and environments, and significant (P≤0.01) genotype x environment (G x E) interactions for all characteristics studied. Due to significant genotypic differences, it was appropriate to continue with subsequent analyses to partition the genotypic variances into parent, parent *vs*. crosses, and crosses components respectively. The crosses variance was partitioned into line, tester and line x tester interaction components making use of a line x tester analysis. The G x E interaction variance was similarly partitioned. All these results were combined in Tables 6.1 to 6.3. Excluding uniformity, less than 17% of the total phenotypic variation was attributed to G x E interaction variation and will, therefore, not be further discussed in this study.

For all 15 plant characteristics evaluated, the mean square (MS) values for parents and crosses were highly significant (Tables 6.1 to 6.3). The percentage variation attributed to different sources of variation was based on the sum of squares. In the case of green-red colour contribution in the leaves (Leaf a*), yellow-blue colour contribution in leaves (Leaf b*) and leaf width (LW), more than 50% of the variation in the genotypes was contributed by the parents (Table 6.1). The parents and crosses respectively contributed 39% and 47% to the total genotypic variation of leaf chlorophyll content (CHL). For Leaf b*, LW and petiole length (PL), variation contributed by the crosses ranged from 36% to 40%. Parents *vs.* crosses (PvsC) contributed less than 32% of total genotypic variation and was not significant in the case of Leaf a* and Leaf b*.

**Table 6.1** Analysis of variance for line x tester analyses and the contribution of main effects within chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL) in butternut across six environments

| | | CHL | | Leaf a* | | Leaf b* | | LW | | PL | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variance | df | MS | % Var | MS | % Var | MS | % Var | MS | % Var | MS | % Var |
| Env | 5 | 2530.22 *** | 49.41 [a] | 1095.68 *** | 67.83 [a] | 1370.14 *** | 66.09 [a] | 40354.97 *** | 41.25 [a] | 101654.47 *** | 47.75 [a] |
| Block(Env) | 12 | 101.30 *** | 4.75 [a] | 14.74 *** | 2.19 [a] | 15.50 *** | 1.79 [a] | 1415.47 *** | 3.47 [a] | 2243.22 *** | 2.53 [a] |
| Genotype | 23 | 259.03 *** | 23.27 [a] | 33.02 *** | 9.40 [a] | 80.11 *** | 17.78 [a] | 7965.29 *** | 37.45 [a] | 13004.30 *** | 28.10 [a] |
| Parent | 7 | 331.47 *** | 38.95 [b] | 70.75 *** | 65.20 [b] | 168.19 *** | 63.89 [b] | 14565.88 *** | 55.66 [b] | 12212.61 *** | 28.58 [b] |
| PvsC | 1 | 861.12 *** | 14.45 [b] | 0.07 NS | 0.01 [b] | 8.54 NS | 0.46 [b] | 9059.17 ** | 4.94 [b] | 92768.83 *** | 31.02 [b] |
| Crosses | 15 | 185.09 *** | 46.60 [b] | 17.62 *** | 34.79 [b] | 43.78 *** | 35.64 [b] | 4812.09 *** | 39.40 [b] | 8056.12 *** | 40.40 [b] |
| Line | 3 | 752.21 *** | 81.28 [c] | 56.69 *** | 64.36 [c] | 88.94 *** | 40.63 [c] | 12509.85 *** | 51.99 [c] | 8175.84 *** | 20.30 [c] |
| Tester | 3 | 76.33 ** | 8.25 [c] | 20.45 ** | 23.21 [c] | 116.33 *** | 53.14 [c] | 10620.74 *** | 44.14 [c] | 29783.95 *** | 73.94 [c] |
| Line x Tester | 9 | 32.30 ** | 10.47 [c] | 3.65 NS | 12.43 [c] | 4.55 NS | 6.23 [c] | 309.96 * | 3.86 [c] | 773.60 * | 5.76 [c] |
| Genotype x Env | 115 | 20.37 ** | 9.15 [a] | 6.87 *** | 9.78 [a] | 5.17 ** | 5.74 [a] | 375.53 *** | 8.83 [a] | 1004.98 *** | 10.86 [a] |
| Parent x Env | 35 | 20.19 * | 30.16 [d] | 10.16 *** | 45.03 [d] | 9.08 ** | 53.42 [d] | 421.15 ** | 34.13 [d] | 972.77 ** | 29.46 [d] |
| PvsC x Env | 5 | 46.92 NS | 10.01 [d] | 10.82 NS | 6.85 [d] | 0.72 NS | 0.60 [d] | 1206.65 NS | 13.97 [d] | 4637.42 ** | 20.06 [d] |
| Crosses x Env | 75 | 18.69 * | 59.83 [d] | 5.07 ** | 48.11 [d] | 3.65 NS | 45.98 [d] | 298.84 ** | 51.90 [d] | 777.84 *** | 50.48 [d] |
| Line x Env | 15 | 29.86 ** | 31.95 [e] | 7.80 ** | 30.81 [e] | 2.68 NS | 14.69 [e] | 529.99 *** | 35.47 [e] | 1816.35 *** | 46.70 [e] |
| Tester x Env | 15 | 16.48 NS | 17.64 [e] | 7.99 ** | 31.54 [e] | 4.95 * | 27.15 [e] | 292.96 * | 19.61 [e] | 787.11 ** | 20.24 [e] |
| Line x Tester x Env | 45 | 15.70 NS | 50.41 [e] | 3.18 NS | 37.66 [e] | 3.53 NS | 58.16 [e] | 223.74 * | 44.92 [e] | 428.58 NS | 33.06 [e] |
| Error | 276 | 12.45 | | 3.16 | | 3.23 | | 159.64 | | 415.15 | |
| Corrected Total | 431 | | | | | | | | | | |

*P≤0.05, **P≤0.01, ***P≤0.001, NS: Not significant, df: Degrees of freedom, MS: Mean squares, % Var: Percentage sum of squares, [a]Expressed as total sum of squares, [b]Expressed as genotype sum of squares, [c]Expressed as crosses sum of squares, [d]Expressed as genotype x environment sum of squares, [e]Expressed as crosses x environment sum of squares, Env: Environment, PvsC: Parents *vs.* crosses

All MS values with regard to genotypic variation (including parent, PvsC, crosses, line, tester and line x tester interactions) were significant for yield and yield components, with the exception of line x tester interaction for fruit number (FN) (Table 6.2). Crosses contributed 62% of the genotypic variation in average fruit mass (AFM). For uniformity and FN, parents and crosses had similar contributions, with PvsC contributing less than 10% of the genotypic variation. More than 50% of the genotypic variation for both yield and dry matter yield (DMY) was attributed by PvsC variation, suggesting a strong presence of heterotic effects (Kose, 2017). The lines were responsible for most of the variation for AFM (94%) and FN (86%) of the crosses. Lines contributed 55% variation of the crosses for yield, with the remainder being divided equally between the testers and line x tester interactions. Around 40% of the DMY variation was attributed to each of the tester and the line x tester interaction effects. Testers and lines both contributed around 40% to the variation in uniformity of the crosses.

MS values of fruit quality characteristics are presented in Table 6.3. The genotypic variation contributed by the parents ranged from 55% to 73% and so are the main source of variation in crosses. More than a third of the variation in total soluble solids (TSS) and internal breakdown (IBD) was attributed to crosses. Furthermore, close to 90% of the variation in crosses for TSS, dry matter content (DMC) and IBD was due to variation in lines. Lines also contributed 71% and 59% of the variation in crosses of Fruit a* and penetrometer reading (PEN) respectively. A large portion (38%) of the variation in crosses was contributed by the testers for PEN only. The contribution from line x tester interaction for fruit quality characteristics was in all cases below 10% and in the case of TSS, DMC and PEN not significant.

**Table 6.2** Analysis of variance for line x tester analyses and the contribution of main effects within average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity and yield in butternut across six environments

| | | AFM | | DMY | | FN | | Uniformity | | Yield | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variance | df | MS | % Var | MS | % Var | MS | % Var | MS | % Var | MS | % Var |
| Env | 5 | 13.49 *** | 31.78 [a] | 612.67 *** | 60.23 [a] | 8310.67 *** | 23.46 [a] | 999.86 *** | 8.38 [a] | 39528.59 *** | 46.32 [a] |
| Block(Env) | 12 | 0.07 NS | 0.42 [a] | 5.45 ** | 1.29 [a] | 135.78 * | 0.92 [a] | 91.20 NS | 1.83 [a] | 494.26 *** | 1.39 [a] |
| Genotype | 23 | 4.64 *** | 50.33 [a] | 44.83 *** | 20.27 [a] | 4342.19 *** | 56.38 [a] | 823.58 *** | 31.74 [a] | 6174.88 *** | 33.28 [a] |
| Parent | 7 | 4.28 *** | 28.04 [b] | 48.75 *** | 33.10 [b] | 5880.17 *** | 41.21 [b] | 1408.63 *** | 52.05 [b] | 4384.90 *** | 21.61 [b] |
| PvsC | 1 | 11.10 *** | 10.39 [b] | 561.25 *** | 54.43 [b] | 7567.42 *** | 7.58 [b] | 504.17 * | 2.66 [b] | 82608.92 *** | 58.17 [b] |
| Crosses | 15 | 4.38 *** | 61.57 [b] | 8.57 *** | 12.47 [b] | 3409.46 *** | 51.21 [b] | 571.85 *** | 45.28 [b] | 1914.60 *** | 20.22 [b] |
| Line | 3 | 20.67 *** | 94.31 [c] | 7.21 ** | 16.83 [c] | 14666.19 *** | 86.03 [c] | 1082.41 *** | 37.86 [c] | 5253.21 *** | 54.88 [c] |
| Tester | 3 | 0.97 *** | 4.41 [c] | 17.14 *** | 40.00 [c] | 2217.51 *** | 13.01 [c] | 1123.15 *** | 39.28 [c] | 2061.41 *** | 21.53 [c] |
| Line x Tester | 9 | 0.09 ** | 1.27 [c] | 6.17 ** | 43.17 [c] | 54.53 NS | 0.96 [c] | 217.90 ** | 22.86 [c] | 752.79 *** | 23.59 [c] |
| Genotype x Env | 115 | 0.22 *** | 12.08 [a] | 4.19 *** | 9.48 [a] | 149.87 *** | 9.73 [a] | 147.88 *** | 28.50 [a] | 395.11 *** | 10.65 [a] |
| Parent x Env | 35 | 0.37 *** | 50.16 [d] | 4.70 *** | 34.10 [d] | 218.39 *** | 44.35 [d] | 159.35 ** | 32.79 [d] | 409.23 *** | 31.52 [d] |
| PvsC x Env | 5 | 0.34 NS | 6.59 [d] | 18.90 *** | 19.60 [d] | 511.17 NS | 14.83 [d] | 398.06 ** | 11.70 [d] | 1703.59 ** | 18.75 [d] |
| Crosses x Env | 75 | 0.15 *** | 43.25 [d] | 2.98 ** | 46.29 [d] | 93.80 ** | 40.82 [d] | 125.85 *** | 55.50 [d] | 301.29 *** | 49.73 [d] |
| Line x Env | 15 | 0.48 *** | 65.12 [e] | 4.22 ** | 28.38 [e] | 192.07 *** | 40.95 [e] | 214.63 *** | 34.11 [e] | 662.24 *** | 43.96 [e] |
| Tester x Env | 15 | 0.14 *** | 19.07 [e] | 3.70 * | 24.88 [e] | 82.37 NS | 17.56 [e] | 165.37 ** | 26.28 [e] | 205.09 NS | 13.61 [e] |
| Line x Tester x Env | 45 | 0.04 NS | 15.81 [e] | 2.32 NS | 46.74 [e] | 64.86 NS | 41.48 [e] | 83.09 NS | 39.61 [e] | 213.04 * | 42.43 [e] |
| Error | 276 | 0.04 | | 1.61 | | 61.08 | | 63.91 | | 129.31 | |
| Corrected Total | 431 | | | | | | | | | | |

*P≤0.05, **P≤0.01, ***P≤0.001, NS: Not significant, df: Degrees of freedom, MS: Mean squares, % Var: Percentage sum of squares, [a]Expressed as total sum of squares, [b]Expressed as genotype sum of squares, [c]Expressed as crosses sum of squares, [d]Expressed as genotype x environment sum of squares, [e]Expressed as crosses x environment sum of squares, Env: Environment, PvsC: Parents *vs.* crosses

**Table 6.3** Analysis of variance for line x tester analyses and the contribution of main effects within total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) in butternut across six environments

| | | TSS | | DMC | | Fruit a* | | IBD | | PEN | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Source of variance | df | MS | % Var | MS | % Var | MS | % Var | MS | % Var | MS | % Var |
| Env | 5 | 29.58 *** | 11.59 [a] | 58.87 *** | 12.81 [a] | 107.38 *** | 6.83 [a] | 2608.04 *** | 9.20 [a] | 3.97 *** | 18.58 [a] |
| Block(Env) | 12 | 0.79 NS | 0.74 [a] | 6.71 *** | 3.50 [a] | 11.38 ** | 1.74 [a] | 345.32 *** | 2.92 [a] | 0.59 *** | 6.67 [a] |
| Genotype | 23 | 33.09 *** | 59.67 [a] | 61.66 *** | 61.70 [a] | 221.82 *** | 64.86 [a] | 3366.64 *** | 54.64 [a] | 2.60 *** | 56.06 [a] |
| Parent | 7 | 59.63 *** | 54.85 [b] | 128.75 *** | 63.55 [b] | 533.70 *** | 73.23 [b] | 6517.82 *** | 58.92 [b] | 5.37 *** | 62.76 [b] |
| PvsC | 1 | 71.02 *** | 9.33 [b] | 134.89 *** | 9.51 [b] | 377.24 *** | 7.39 [b] | 2527.31 ** | 3.26 [b] | 6.21 *** | 10.37 [b] |
| Crosses | 15 | 18.17 *** | 35.82 [b] | 25.47 *** | 26.94 [b] | 65.91 *** | 19.38 [b] | 1952.04 *** | 37.81 [b] | 1.07 *** | 26.86 [b] |
| Line | 3 | 83.21 *** | 91.59 [c] | 117.46 *** | 92.23 [c] | 233.88 *** | 70.97 [c] | 8613.16 *** | 88.25 [c] | 3.16 *** | 58.88 [c] |
| Tester | 3 | 5.92 *** | 6.52 [c] | 6.50 ** | 5.10 [c] | 74.49 *** | 22.60 [c] | 572.31 *** | 5.86 [c] | 2.04 *** | 38.09 [c] |
| Line x Tester | 9 | 0.57 NS | 1.89 [c] | 1.13 NS | 2.66 [c] | 7.06 * | 6.43 [c] | 191.57 ** | 5.89 [c] | 0.05 NS | 3.02 [c] |
| Genotype x Env | 115 | 1.47 *** | 13.25 [a] | 2.02 *** | 10.09 [a] | 8.04 *** | 11.76 [a] | 205.76 *** | 16.70 [a] | 0.09 *** | 9.55 [a] |
| Parent x Env | 35 | 2.18 ** | 45.05 [d] | 2.60 ** | 39.17 [d] | 10.09 * | 38.18 [d] | 199.99 * | 29.58 [d] | 0.14 *** | 48.74 [d] |
| PvsC x Env | 5 | 1.80 NS | 5.33 [d] | 1.67 NS | 3.60 [d] | 18.55 NS | 10.03 [d] | 251.86 NS | 5.32 [d] | 0.30 NS | 14.87 [d] |
| Crosses x Env | 75 | 1.12 ** | 49.62 [d] | 1.77 ** | 57.23 [d] | 6.39 ** | 51.79 [d] | 205.38 *** | 65.10 [d] | 0.05 ** | 36.39 [d] |
| Line x Env | 15 | 2.02 *** | 36.13 [e] | 3.27 *** | 37.00 [e] | 14.91 *** | 46.69 [e] | 547.77 *** | 53.34 [e] | 0.08 ** | 32.70 [e] |
| Tester x Env | 15 | 1.35 ** | 24.14 [e] | 1.99 ** | 22.49 [e] | 7.10 * | 22.22 [e] | 108.97 NS | 10.61 [e] | 0.10 *** | 39.07 [e] |
| Line x Tester x Env | 45 | 0.74 NS | 39.73 [e] | 1.19 NS | 40.51 [e] | 3.31 NS | 31.09 [e] | 123.39 ** | 36.05 [e] | 0.02 NS | 28.24 [e] |
| Error | 276 | 0.68 | | 0.99 | | 4.22 | | 84.92 | | 0.04 | |
| Corrected Total | 431 | | | | | | | | | | |

*P≤0.05, **P≤0.01, ***P≤0.001, NS: Not significant, df: Degrees of freedom, MS: Mean squares, % Var: Percentage sum of square, [a]Expressed as total sum of squares, [b]Expressed as genotype sum of squares, [c]Expressed as crosses sum of squares, [d]Expressed as genotype x environment sum of squares, e: Expressed as crosses x environment sum of squares, Env: Environment, PvsC: Parents *vs.* crosses

Various genetic components were estimated based on MS values for all 15 of the characteristics evaluated (Table 6.4). However, since the MS values of line x tester interactions for Leaf a*, Leaf b*, FN, TSS, DMC and PEN were not significant, it would have not been appropriate to use these estimates for the calculation of SCA variance and all subsequent formulae requiring this value (Shams *et al*., 2010). The SCA variance ($\sigma^2_{SCA}$) was calculated to be higher than the GCA variance ($\sigma^2_{GCA}$) for CHL, DMY, uniformity and yield; suggesting that these traits were most likely under non-additive gene action. In contrast, the predictability ratio ($\sigma^2_{GCA}/\sigma^2_{SCA}$) was larger than one for LW, PL, AFM, Fruit a* and IBD; suggesting mostly additive gene action. Narrow-sense heritability ($h^2_{ns}$) and broad-sense heritability ($h^2_{bs}$) ranged from 0.33 to 0.98 and 0.82 to 0.99, respectively. The degree of dominance $(\sigma^2_D/\sigma^2_A)^{1/2}$ was smaller than one for CHL, LW, PL, AFM, Fruit a* and IBD; suggesting mostly additive gene action. DMY had a high value (2.1), which suggested the prevalence of dominance variance. For both uniformity and yield, the ratio was close to one and suggested that both additive and dominant gene action was important.

GCA effects are useful in selecting the best lines as potential parents for desirable hybrid combinations. It was reported (Istipliler *et al*., 2015; Kose, 2017) that when significant differences in MS values are observed for lines, testers and crosses, it is appropriate to calculate GCA and SCA effects for characteristics. Combining abilities were estimated from combined means across the six environments for the traits of interest and presented in Tables 6.5 to 6.7. Both beneficial and unfavourable effects, which were significant, were indicated in green and orange in these tables respectively.

There were significant differences among the GCA effects of the lines and the testers for all the characteristics measured. With respect to CHL, G3 and G6 were the only parents with significant positive effects (Table 6.5). Since lower mean values are more beneficial when it comes to Leaf a*, it is therefore valuable to mention the negative significant effects of G7 and G8 that were estimated. Of the testers, only G4 had a significant effect for Leaf a*. Highly significant positive GCA effects were observed in Leaf b* for G1, G2 and G8. Positive and high GCA effects were recorded for G4 and G5 for LW, while G8 was on the opposite end of the range with a high negative GCA effect. PL was positively influenced by G1, G2, G7 and G8.

**Table 6.4** Estimates of genetic components for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity and yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) in butternut across six environments

| Estimate | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniformity | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $\sigma^2_{GCA}$ | 0.88 | 0.08 | 0.23 | 26.05 | 42.14 | 0.02 | 0.01 | 19.42 | 2.05 | 6.72 | 0.10 | 0.14 | 0.34 | 10.19 | 0.01 |
| $\sigma^2_{SCA}$ | 1.10 | | | 8.35 | 19.91 | 0.00 | 0.25 | | 8.56 | 34.64 | | | 0.16 | 5.93 | |
| $\sigma^2_{GCA}/\sigma^2_{SCA}$ | 0.80 | | | 3.12 | 2.12 | 8.68 | 0.05 | | 0.24 | 0.19 | | | 2.16 | 1.72 | |
| $\sigma^2_A$ | 3.54 | | | 104.22 | 168.58 | 0.10 | 0.06 | | 8.19 | 26.89 | | | 1.36 | 40.75 | |
| $\sigma^2_D$ | 1.10 | | | 8.35 | 19.91 | 0.00 | 0.25 | | 8.56 | 34.64 | | | 0.16 | 5.93 | |
| $(\sigma^2_D/\sigma^2_A)^{1/2}$ | 0.56 | | | 0.28 | 0.34 | 0.17 | 2.13 | | 1.02 | 1.13 | | | 0.34 | 0.38 | |
| $\sigma^2_e$ | 0.69 | | | 8.87 | 23.06 | 0.00 | 0.09 | | 3.55 | 7.18 | | | 0.23 | 4.72 | |
| $h^2_{ns}$ | 0.86 | | | 0.95 | 0.92 | 0.98 | 0.33 | | 0.67 | 0.66 | | | 0.91 | 0.92 | |
| $h^2_{bs}$ | 0.94 | | | 0.97 | 0.96 | 0.99 | 0.82 | | 0.90 | 0.94 | | | 0.95 | 0.96 | |

$\sigma^2_{GCA}$: GCA variance, $\sigma^2_{SCA}$: SCA variance, $\sigma^2_{GCA}/\sigma^2_{SCA}$: Predictability ratio, $\sigma^2_A$: Additive variance, $\sigma^2_D$: Dominance variance, $(\sigma^2_D/\sigma^2_A)^{1/2}$: Degree of dominance, $\sigma^2_e$: Error variance, $h^2_{ns}$: Narrow-sense heritability, $h^2_{bs}$: Broad-sense heritability

**Table 6.5** General and specific combining abilities for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL) in butternut across six environments

| Entry | Genotype | CHL | Leaf a* | Leaf b* | LW | PL |
|---|---|---|---|---|---|---|
| General combining ability | | | | | | |
| Testers | | | | | | |
| G1 | BUT01 | 0.40 NS | -0.14 NS | 0.96 *** | -11.58 *** | 11.62 *** |
| G2 | BUT02 | -1.21 ** | -0.36 NS | 0.92 *** | -7.36 *** | 18.80 *** |
| G3 | BUT03 | 1.19 ** | -0.29 NS | -0.13 NS | 3.35 * | -3.16 NS |
| G4 | BUT04 | -0.38 NS | 0.79 *** | -1.75 *** | 15.59 *** | -27.25 *** |
| $SE_t$ | | 1.66 | 0.84 | 0.85 | 5.96 | 9.60 |
| Lines | | | | | | |
| G5 | BUT05 | -3.51 *** | 0.14 NS | -0.46 * | 10.40 *** | -13.09 *** |
| G6 | BUT06 | 4.06 *** | 1.20 *** | -1.26 *** | 1.09 NS | -3.80 NS |
| G7 | BUT07 | -1.35 ** | -0.64 ** | 0.40 NS | 7.41 *** | 10.91 *** |
| G8 | BUT08 | 0.80 NS | -0.70 ** | 1.32 *** | -18.90 *** | 5.98 * |
| $SE_l$ | | 1.66 | 0.84 | 0.85 | 5.96 | 9.60 |
| Specific combining ability | | | | | | |
| Crosses | | | | | | |
| G9 | BUT01xBUT05 | 0.27 NS | 0.33 NS | -0.22 NS | -4.97 NS | -0.18 NS |
| G10 | BUT02xBUT05 | -0.10 NS | -0.83 * | 0.79 NS | -2.58 NS | 7.91 NS |
| G11 | BUT03xBUT05 | 1.52 NS | -0.17 NS | 0.29 NS | 5.15 NS | -1.13 NS |
| G12 | BUT04xBUT05 | -1.68 * | 0.67 NS | -0.86 * | 2.39 NS | -6.59 NS |
| G13 | BUT01xBUT06 | -0.33 NS | 0.01 NS | 0.09 NS | 3.40 NS | 8.41 NS |
| G14 | BUT02xBUT06 | -0.26 NS | 0.27 NS | -0.19 NS | 0.07 NS | -7.32 NS |
| G15 | BUT03xBUT06 | 0.72 NS | -0.02 NS | -0.14 NS | 1.69 NS | 0.41 NS |
| G16 | BUT04xBUT06 | -0.13 NS | -0.27 NS | 0.25 NS | -5.16 NS | -1.50 NS |
| G17 | BUT01xBUT07 | 1.37 NS | -0.04 NS | -0.17 NS | 0.41 NS | -0.24 NS |
| G18 | BUT02xBUT07 | -0.81 NS | 0.29 NS | -0.36 NS | -0.70 NS | -0.20 NS |
| G19 | BUT03xBUT07 | -1.00 NS | 0.12 NS | -0.05 NS | -1.11 NS | 1.54 NS |
| G20 | BUT04xBUT07 | 0.44 NS | -0.36 NS | 0.58 NS | 1.40 NS | -1.09 NS |
| G21 | BUT01xBUT08 | -1.31 NS | -0.30 NS | 0.31 NS | 1.16 NS | -7.98 NS |
| G22 | BUT02xBUT08 | 1.17 NS | 0.27 NS | -0.24 NS | 3.21 NS | -0.39 NS |
| G23 | BUT03xBUT08 | -1.24 NS | 0.08 NS | -0.11 NS | -5.74 NS | -0.81 NS |
| G24 | BUT04xBUT08 | 1.37 NS | -0.04 NS | 0.03 NS | 1.37 NS | 9.19 NS |
| $SE_c$ | | 0.83 NS | 0.42 NS | 0.42 NS | 2.98 NS | 4.80 NS |

$SE_l$: Standard error for lines, $SE_t$: Standard error for testers, $SE_c$: Standard error for crosses; Significant beneficial and unfavourable effects are highlighted in green and orange respectively

Genotypes G5 and G6 had the largest positive and negative GCA effects respectively on AFM (Table 6.6). Positive effects of DMY were recorded for both G1 and G6 and the same two parents plus G8 had a positive impact on FN. Uniformity was positively impacted by G3, G4 and G8, while G1, G2, G5 and G8 all contributed towards higher yields. The two lines, G6 and G7, showed to have a highly significant and positive impact on all the internal fruit quality characteristics (Table 6.7). G8 resulted in highly significant negative effects only, with an extremely high negative effect for IBD. Of the testers, G3 positively affected TSS, DMC and PEN. The characteristic Fruit a* was positively impacted by G2 and G4, while G4 positively affected IBD.

The SCA effects were not significant across all crosses for LW, PL, FN, TSS, DMC and PEN (Tables 6.5 to 6.7). Only a limited number of significant SCA effects were observed across all characteristics, and many of these were negative. The only significant positive SCA effects were the crosses G9 for uniformity and yield, G10 for AFM, G16 for yield, and both G11 and G24 for IBD.

Heterosis estimates can be attributed to both additive and non-additive gene actions (Fellahi *et al*., 2013). All characteristics evaluated in this study showed significant heterosis estimated from the means combined across all six environments (Tables 6.8 to 6.10).

The heterosis percentages for CHL, Leaf a*, Leaf b*, LW and PL are presented in Table 6.8. The minimum and maximum heterosis percentages across the plant characteristics ranged from 10.5% as the smallest (LW) to 17.9% as the largest (CHL). The average heterosis ranged from -0.9% for Leaf b* to 10.0% for PL. The genotypes with the highest heterosis were G11 and G24, with 17.6% for CHL and 16.4% for PL, respectively. Leaf a* and Leaf b* showed both significant positive and negative heterosis across the 16 crosses.

The heterosis percentages for the yield characteristics are presented in Table 6.9. In general, yield and yield components displayed the highest levels of heterosis. The smallest range of heterosis within each characteristic was 23.5% for uniformity and the largest 77.9% for yield. The average heterosis for FN, AFM, DMY and yield was 20.9%, 23.2%, 44.1% and 58.3%, respectively. The highest heterosis was measured in G9 and G10 with values greater than 100% for yield.

**Table 6.6** General and specific combining abilities for average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity and yield in butternut across six environments

| Entry | Genotype | AFM | DMY | FN | Uniformity | Yield |
|---|---|---|---|---|---|---|
| General combining ability | | | | | | |
| Testers | | | | | | |
| G1 | BUT01 | -0.12 *** | 0.48 ** | 5.71 *** | -2.92 ** | 3.85 ** |
| G2 | BUT02 | 0.07 ** | 0.01 NS | 0.53 NS | -3.89 *** | 4.10 ** |
| G3 | BUT03 | -0.07 ** | 0.19 NS | 1.35 NS | 3.33 *** | -0.61 NS |
| G4 | BUT04 | 0.12 *** | -0.67 *** | -7.59 *** | 3.47 *** | -7.34 *** |
| $SE_t$ | | 0.10 | 0.60 | 3.68 | 3.77 | 5.36 |
| Lines | | | | | | |
| G5 | BUT05 | 0.73 *** | -0.27 NS | -14.36 *** | -4.03 *** | 5.05 *** |
| G6 | BUT06 | -0.50 *** | 0.46 ** | 12.98 *** | -0.28 NS | -2.11 NS |
| G7 | BUT07 | 0.04 NS | -0.11 NS | -10.16 *** | -0.97 NS | -11.08 *** |
| G8 | BUT08 | -0.27 *** | -0.07 NS | 11.55 *** | 5.28 *** | 8.14 *** |
| $SE_l$ | | 0.10 | 0.60 | 3.68 | 3.77 | 5.36 |
| Specific combining ability | | | | | | |
| Crosses | | | | | | |
| G9 | BUT01xBUT05 | 0.02 NS | 0.48 NS | -0.56 NS | 5.83 ** | 6.53 * |
| G10 | BUT02xBUT05 | 0.11 * | 0.43 NS | 0.01 NS | 1.81 NS | 4.27 NS |
| G11 | BUT03xBUT05 | -0.11 * | 0.17 NS | 2.41 NS | -5.42 ** | 2.01 NS |
| G12 | BUT04xBUT05 | -0.02 NS | -1.09 *** | -1.86 NS | -2.22 NS | -12.82 *** |
| G13 | BUT01xBUT06 | 0.00 NS | -0.40 NS | -0.17 NS | -0.14 NS | -4.04 NS |
| G14 | BUT02xBUT06 | -0.08 NS | -0.37 NS | 0.18 NS | -1.94 NS | -3.73 NS |
| G15 | BUT03xBUT06 | 0.05 NS | 0.26 NS | -0.81 NS | 1.39 NS | -0.92 NS |
| G16 | BUT04xBUT06 | 0.04 NS | 0.51 NS | 0.80 NS | 0.69 NS | 8.69 ** |
| G17 | BUT01xBUT07 | -0.01 NS | 0.20 NS | 0.08 NS | -3.89 * | 0.18 NS |
| G18 | BUT02xBUT07 | 0.03 NS | 0.25 NS | -0.41 NS | 0.42 NS | 0.94 NS |
| G19 | BUT03xBUT07 | -0.01 NS | -0.53 NS | -2.34 NS | 3.19 NS | -3.75 NS |
| G20 | BUT04xBUT07 | -0.01 NS | 0.09 NS | 2.66 NS | 0.28 NS | 2.63 NS |
| G21 | BUT01xBUT08 | -0.01 NS | -0.28 NS | 0.65 NS | -1.81 NS | -2.67 NS |
| G22 | BUT02xBUT08 | -0.06 NS | -0.31 NS | 0.22 NS | -0.28 NS | -1.49 NS |
| G23 | BUT03xBUT08 | 0.08 NS | 0.11 NS | 0.73 NS | 0.83 NS | 2.66 NS |
| G24 | BUT04xBUT08 | -0.01 NS | 0.49 NS | -1.60 NS | 1.25 NS | 1.50 NS |
| $SE_c$ | | 0.05 NS | 0.30 NS | 1.84 NS | 1.88 NS | 2.68 NS |

$SE_l$: Standard error for lines, $SE_t$: Standard error for testers, $SE_c$: Standard error for crosses; Significant beneficial and unfavourable effects are highlighted in green and orange respectively

**Table 6.7** General and specific combining abilities for total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) in butternut across six environments

| Entry | Genotype | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|
| General combining ability | | | | | | |
| Testers | | | | | | |
| G1 | BUT01 | 0.02 NS | 0.11 NS | -1.24 *** | 1.11 NS | -0.08 *** |
| G2 | BUT02 | -0.41 *** | -0.38 ** | 0.53 * | -3.85 *** | -0.08 *** |
| G3 | BUT03 | 0.22 * | 0.33 ** | -0.36 NS | -0.05 NS | 0.25 *** |
| G4 | BUT04 | 0.17 NS | -0.06 NS | 1.08 *** | 2.79 * | -0.09 *** |
| $SE_t$ | | 0.39 | 0.47 | 0.97 | 4.34 | 0.09 |
| Lines | | | | | | |
| G5 | BUT05 | -0.91 *** | -1.07 *** | -2.01 *** | 2.33 * | -0.06 ** |
| G6 | BUT06 | 0.56 *** | 0.71 *** | 1.00 *** | 6.09 *** | 0.19 *** |
| G7 | BUT07 | 1.24 *** | 1.44 *** | 1.95 *** | 7.65 *** | 0.14 *** |
| G8 | BUT08 | -0.89 *** | -1.09 *** | -0.95 *** | -16.06 *** | -0.27 *** |
| $SE_l$ | | 0.39 | 0.47 | 0.97 | 4.34 | 0.09 |
| Specific combining ability | | | | | | |
| Crosses | | | | | | |
| G9 | BUT01xBUT05 | -0.04 NS | -0.03 NS | 0.34 NS | -0.65 NS | 0.02 NS |
| G10 | BUT02xBUT05 | 0.03 NS | 0.06 NS | 0.34 NS | -1.18 NS | -0.02 NS |
| G11 | BUT03xBUT05 | -0.05 NS | -0.13 NS | 0.59 NS | 5.23 * | -0.03 NS |
| G12 | BUT04xBUT05 | 0.06 NS | 0.10 NS | -1.27 ** | -3.40 NS | 0.03 NS |
| G13 | BUT01xBUT06 | -0.17 NS | -0.04 NS | -0.27 NS | 1.07 NS | -0.06 NS |
| G14 | BUT02xBUT06 | 0.07 NS | 0.12 NS | 0.34 NS | 1.37 NS | -0.03 NS |
| G15 | BUT03xBUT06 | 0.23 NS | 0.32 NS | -0.40 NS | -1.97 NS | 0.07 NS |
| G16 | BUT04xBUT06 | -0.12 NS | -0.41 NS | 0.33 NS | -0.47 NS | 0.02 NS |
| G17 | BUT01xBUT07 | 0.04 NS | 0.12 NS | 0.24 NS | -1.13 NS | 0.03 NS |
| G18 | BUT02xBUT07 | -0.03 NS | -0.02 NS | -0.45 NS | 0.39 NS | -0.04 NS |
| G19 | BUT03xBUT07 | 0.10 NS | 0.00 NS | -0.20 NS | 1.44 NS | 0.00 NS |
| G20 | BUT04xBUT07 | -0.11 NS | -0.09 NS | 0.41 NS | -0.70 NS | 0.01 NS |
| G21 | BUT01xBUT08 | 0.17 NS | -0.05 NS | -0.30 NS | 0.71 NS | 0.00 NS |
| G22 | BUT02xBUT08 | -0.06 NS | -0.16 NS | -0.23 NS | -0.57 NS | 0.09 NS |
| G23 | BUT03xBUT08 | -0.29 NS | -0.19 NS | 0.00 NS | -4.71 * | -0.04 NS |
| G24 | BUT04xBUT08 | 0.18 NS | 0.40 NS | 0.53 NS | 4.57 * | -0.05 NS |
| $SE_c$ | | 0.19 NS | 0.23 NS | 0.48 NS | 2.17 NS | 0.04 NS |

$SE_l$: Standard error for lines, $SE_t$: Standard error for testers, $SE_c$: Standard error for crosses; Significant beneficial and unfavourable effects are highlighted in green and orange respectively

**Table 6.8** Heterosis (%) for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL) in butternut across six environments

| Entry | Genotype | CHL | Leaf a* | Leaf b* | LW | PL |
|---|---|---|---|---|---|---|
| G9 | BUT01xBUT05 | 8.3 ** | 3.8 NS | 2.7 NS | -2.5 NS | 6.7 *** |
| G10 | BUT02xBUT05 | 10.1 ** | 6.6 * | 4.4 * | 0.2 NS | 13.0 *** |
| G11 | BUT03xBUT05 | 17.6 *** | 2.3 NS | 1.1 NS | 1.6 NS | 7.0 *** |
| G12 | BUT04xBUT05 | 4.4 NS | 6.8 * | 4.5 NS | 1.4 NS | 5.3 * |
| G13 | BUT01xBUT06 | 7.3 ** | -4.6 NS | -2.7 NS | 3.3 * | 11.7 *** |
| G14 | BUT02xBUT06 | 9.8 *** | -8.6 ** | -5.7 ** | 4.1 ** | 10.4 *** |
| G15 | BUT03xBUT06 | 14.5 *** | -8.0 ** | -7.1 *** | 3.2 * | 9.9 *** |
| G16 | BUT04xBUT06 | 9.0 *** | 1.0 NS | 1.5 NS | 1.5 NS | 9.7 *** |
| G17 | BUT01xBUT07 | 6.9 * | 0.3 NS | -2.0 NS | 4.8 *** | 7.2 *** |
| G18 | BUT02xBUT07 | 3.6 NS | -4.0 NS | -4.6 * | 6.4 *** | 10.6 *** |
| G19 | BUT03xBUT07 | 5.9 * | -4.0 NS | -4.9 * | 4.8 *** | 8.3 *** |
| G20 | BUT04xBUT07 | 5.9 * | 6.3 * | 4.5 * | 6.2 *** | 7.7 *** |
| G21 | BUT01xBUT08 | -0.2 NS | 2.9 NS | -0.1 NS | 4.9 ** | 9.2 *** |
| G22 | BUT02xBUT08 | 7.9 ** | -2.8 NS | -3.8 * | 8.0 *** | 15.2 *** |
| G23 | BUT03xBUT08 | 4.6 NS | -2.7 NS | -4.8 * | 2.8 NS | 12.2 *** |
| G24 | BUT04xBUT08 | 7.4 ** | 6.0 * | 2.5 NS | 6.1 *** | 16.4 *** |
| Average | | 7.7 | 0.1 | -0.9 | 3.5 | 10.0 |
| Minimum | | -0.2 | -8.6 | -7.1 | -2.5 | 5.3 |
| Maximum | | 17.6 | 6.8 | 4.5 | 8.0 | 16.4 |
| Range | | 17.9 | 15.4 | 11.6 | 10.5 | 11.1 |

*P≤0.05, **P≤0.01, ***P≤0.001, NS: Not significant

**Table 6.9** Heterosis (%) for average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity and yield in butternut across six environments

| Entry | Genotype | AFM | DMY | FN | Uniformity | Yield |
|---|---|---|---|---|---|---|
| G9 | BUT01xBUT05 | 37.0 *** | 84.3 *** | 11.0 NS | 12.8 ** | 101.7 *** |
| G10 | BUT02xBUT05 | 45.1 *** | 87.9 *** | 18.6 * | 10.2 * | 106.9 *** |
| G11 | BUT03xBUT05 | 26.0 *** | 71.1 *** | 30.1 *** | -10.7 * | 97.8 *** |
| G12 | BUT04xBUT05 | 32.8 *** | 59.7 *** | 3.6 NS | -7.1 NS | 52.4 *** |
| G13 | BUT01xBUT06 | 6.1 NS | 31.5 *** | 37.3 *** | 9.6 * | 46.5 *** |
| G14 | BUT02xBUT06 | 6.9 NS | 32.1 *** | 45.9 *** | 11.5 * | 53.7 *** |
| G15 | BUT03xBUT06 | 7.4 NS | 36.4 *** | 46.3 *** | 8.8 * | 54.4 *** |
| G16 | BUT04xBUT06 | 11.8 * | 49.2 *** | 46.2 *** | 5.3 NS | 63.6 *** |
| G17 | BUT01xBUT07 | 15.3 *** | 29.4 *** | 5.8 NS | -3.8 NS | 35.9 *** |
| G18 | BUT02xBUT07 | 23.1 *** | 30.3 *** | 8.4 NS | 9.8 * | 43.5 *** |
| G19 | BUT03xBUT07 | 12.5 ** | 14.0 * | 6.1 NS | 6.6 NS | 29.0 *** |
| G20 | BUT04xBUT07 | 16.1 *** | 28.9 *** | 10.3 NS | -0.5 NS | 31.0 *** |
| G21 | BUT01xBUT08 | 32.4 *** | 33.4 *** | 15.1 *** | 2.5 NS | 49.3 *** |
| G22 | BUT02xBUT08 | 33.6 *** | 33.3 *** | 18.1 *** | 9.9 * | 57.4 *** |
| G23 | BUT03xBUT08 | 34.5 *** | 34.4 *** | 21.1 *** | 4.4 NS | 60.4 *** |
| G24 | BUT04xBUT08 | 29.8 *** | 50.2 *** | 10.1 * | 3.0 NS | 49.2 *** |
| Average | | 23.2 | 44.1 | 20.9 | 4.5 | 58.3 |
| Minimum | | 6.1 | 14.0 | 3.6 | -10.7 | 29.0 |
| Maximum | | 45.1 | 87.9 | 46.3 | 12.8 | 106.9 |
| Range | | 39.0 | 74.0 | 42.7 | 23.5 | 77.9 |

*P≤0.05, **P≤0.01, ***P≤0.001, NS: Not significant

Higher levels of heterosis were evident in fruit quality characteristics (Table 6.10) where heterosis was the lowest for PEN (20.4%) and the highest for Fruit a* (39.6%). The average heterosis ranged from -9.8% for DMC to 10.4% for PEN. The highest positive heterosis was observed for G21 and G22 with 27.8% and 23.0% respectively, both for IBD. The highest negative heterosis was observed for G21, G22 and G23 of around -26.5% for Fruit a*. Fruit a* was the only quality characteristic that showed both significant positive and negative heterosis across the 16 crosses. Measurements of both TSS and DMC showed significant negative heterosis only.

**Table 6.10** Heterosis (%) for total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) in butternut across six environments

| Entry | Genotype | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|
| G9 | BUT01xBUT05 | -5.4 NS | -3.5 NS | 13.1 ** | 7.9 * | 17.2 *** |
| G10 | BUT02xBUT05 | -9.2 ** | -6.3 * | 6.1 NS | 5.0 NS | 16.1 *** |
| G11 | BUT03xBUT05 | -9.9 ** | -7.9 ** | 8.4 * | 6.6 NS | 7.7 *** |
| G12 | BUT04xBUT05 | 2.7 NS | 4.9 NS | 6.5 NS | -4.4 NS | 18.6 *** |
| G13 | BUT01xBUT06 | -11.0 *** | -15.1 *** | -8.4 * | 11.9 ** | 13.3 *** |
| G14 | BUT02xBUT06 | -12.1 *** | -16.7 *** | -8.3 ** | 10.4 ** | 15.4 *** |
| G15 | BUT03xBUT06 | -10.1 *** | -14.4 *** | -11.9 *** | -0.6 NS | 11.1 *** |
| G16 | BUT04xBUT06 | -5.4 * | -13.8 *** | -0.9 NS | 1.0 NS | 17.3 *** |
| G17 | BUT01xBUT07 | -8.6 *** | -9.2 *** | -8.7 ** | 8.9 * | 3.7 NS |
| G18 | BUT02xBUT07 | -12.7 *** | -13.0 *** | -14.0 *** | 9.0 * | 1.6 NS |
| G19 | BUT03xBUT07 | -11.0 *** | -12.3 *** | -13.3 *** | 3.6 NS | -1.8 NS |
| G20 | BUT04xBUT07 | -5.4 * | -6.3 * | -3.7 NS | 0.8 NS | 3.6 NS |
| G21 | BUT01xBUT08 | -11.5 *** | -11.2 *** | -26.3 *** | 27.8 *** | 12.1 *** |
| G22 | BUT02xBUT08 | -18.8 *** | -15.9 *** | -26.5 *** | 23.0 *** | 16.7 *** |
| G23 | BUT03xBUT08 | -20.4 *** | -15.3 *** | -26.3 *** | 3.1 NS | 3.5 NS |
| G24 | BUT04xBUT08 | -5.8 NS | -0.9 NS | -17.2 *** | 18.8 *** | 10.3 *** |
| Average | | -9.7 | -9.8 | -8.2 | 8.3 | 10.4 |
| Minimum | | -20.4 | -16.7 | -26.5 | -4.4 | -1.8 |
| Maximum | | 2.7 | 4.9 | 13.1 | 27.8 | 18.6 |
| Range | | 23.1 | 21.6 | 39.6 | 32.1 | 20.4 |

*P≤0.05, **P≤0.01, ***P≤0.001, NS: Not significant

The relationships between heterosis, GCA effects, SCA effects and $F_1$ hybrid performance were estimated using simple linear correlation coefficients (Table 6.11). Significant positive correlations were observed between heterosis and the sum of the parental GCA effects (H-$GCA_{Sum}$) for AFM, FN and yield while a significant negative correlation was observed for IBD. Significant positive correlations were observed between heterosis and SCA (H-SCA) for CHL, PL and uniformity. The $F_1$ hybrid performance showed highly significant correlations with the sum of the parental GCA effects ($F_1$-$GCA_{Sum}$) for all the characteristics. Of the correlations between $F_1$ hybrid performance and SCA effects ($F_1$-SCA), only that of DMY was significant. Significant positive correlations were also observed between $F_1$ hybrid performance and heterosis ($F_1$-H) for AFM, FN and yield, while the correlation for IBD was negative.

**Table 6.11** Correlation between heterosis and combining ability for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity and yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD) and mesocarp penetrometer reading (PEN) in butternut across six environments

| Characteristic | H-$GCA_{Sum}$ | H-SCA | $F_1$-$GCA_{Sum}$ | $F_1$-SCA | $F_1$-H |
|---|---|---|---|---|---|
| CHL | 0.11 NS | 0.64 ** | 0.95 *** | 0.32 NS | 0.31 NS |
| Leaf a* | -0.01 NS | -0.32 NS | 0.94 *** | 0.35 NS | -0.12 NS |
| Leaf b* | -0.34 NS | 0.34 NS | 0.97 *** | 0.25 NS | -0.24 NS |
| LW | -0.32 NS | 0.44 NS | 0.98 *** | 0.20 NS | -0.23 NS |
| PL | 0.27 NS | 0.56 * | 0.97 *** | 0.24 NS | 0.40 NS |
| AFM | 0.62 * | 0.27 NS | 0.99 *** | 0.12 NS | 0.64 ** |
| DMY | -0.28 NS | 0.37 NS | 0.75 *** | 0.66 ** | 0.03 NS |
| FN | 0.59 * | 0.27 NS | 1.00 *** | 0.10 NS | 0.61 * |
| Uniformity | -0.25 NS | 0.71 ** | 0.88 *** | 0.48 NS | 0.12 NS |
| Yield | 0.60 * | 0.44 NS | 0.87 *** | 0.49 NS | 0.74 *** |
| TSS | 0.11 NS | 0.32 NS | 0.99 *** | 0.14 NS | 0.15 NS |
| DMC | -0.38 NS | 0.35 NS | 0.99 *** | 0.16 NS | -0.31 NS |
| Fruit a* | -0.20 NS | 0.19 NS | 0.97 *** | 0.25 NS | -0.15 NS |
| IBD | -0.68 ** | 0.43 NS | 0.97 *** | 0.24 NS | -0.56 * |
| PEN | -0.44 NS | 0.27 NS | 0.98 *** | 0.18 NS | -0.39 NS |

*P≤0.05, **P≤0.01, ***P≤0.001, NS: Not significant, H: heterosis, $GCA_{Sum}$: Sum of general combining ability effects of two parents, SCA: Specific combining ability, $F_1$: Performance of the $F_1$ hybrid

### 6.5 Discussion

Significant differences between the lines, testers and crosses confirmed the existence of genotypic variation in most of the characteristics studied, and these can potentially be utilised in breeding programmes for the successful genetic improvement of butternut. The best performing parents can be selected to form part of future breeding activities or can be used as parents for future $F_1$ hybrids. The significant differences among the crosses for some of the traits allow the identification of the most desirable genotypes for commercialisation (Abrha *et al*., 2013).

With the exception of Leaf a* and Leaf b*, significant PvsC effects indicated the existence of hybrid vigour in all the characteristics explored in this study. For yield and DMY, and to a lesser

extend PL, a high proportion of the total genotypic variation (based on the sum of squares) can be attributed to the PvsC component, indicating that a higher level of heterosis could be expected for these characteristics. Later generation selection and hybrid breeding for improvement of these traits would be beneficial (Fellahi *et al*., 2013; Fasahat *et al*., 2016).

Partitioning of the variance component for crosses indicated that lines, testers and line x tester interactions were significant sources of variation for CHL, LW, PL, AFM, DMY, uniformity, yield, Fruit a* and IBD, for which both additive and non-additive gene action will play a significant role in their control (Abrha *et al.*, 2013). This is supported by examples in literature where AFM and yield have been reported to be under both additive and non-additive gene action (Abdein *et al*., 2017; Ahmed *et al*., 2017; Mohsin *et al*., 2017).

Significant line x tester interactions also indicate that different testers will result in different rankings of lines based on their hybrid performance, and it is an indication of predominance of dominance gene action (El-Hosary and Elgammaal, 2013). This is especially appllicable to DMY, where 43% of the variation within crosses could be attributed to line x tester interactions, and was confirmed by the lowest association between the sum of the GCA effects of the parents and *per se* hybrid performance (to be discussed later). Characteristics, for which MS of the lines and testers only were significant, can be regarded as being mostly under additive genetic control and these included Leaf a*, Leaf b*, FN, TSS, DMC and PEN.

Typically, the predictability ratio ($\sigma^2_{GCA}/\sigma^2_{SCA}$) is an indication of the type of gene action involved in the control of the characteristic. In this study the predictability ratio for CHL, DMY, uniformity and yield was calculated as less than one and it would, therefore, be reasonable to assume that $\sigma^2_{SCA}$ is much greater than $\sigma^2_{GCA}$. This indicates the preponderance of non-additive gene action (Fellahi *et al*., 2013). This is especially relevant to DMY, uniformity and yield where this ratio was smaller than 0.25. For traits like these, later generation selection of superior individuals becomes more efficient when making selections among the recombinants within a segregating population. The predictability ratios for LW, PL, AFM, Fruit a* and IBD were all larger than one, indicating these traits to be mostly under additive genetic control. Performance of the hybrids can be predicted based on GCA effects alone, especially for AFM where a predictability ratio close to nine was calculated. Based on $(\sigma^2_D/\sigma^2_A)^{1/2}$, dominance variance played a crucial part (Fellahi *et al*., 2013) in DMY but was of lesser importance for CHL, LW, PL, AFM, Fruit a* and IBD. The degree of dominance for uniformity and yield were close to one, which suggested that that both additive and dominance variance were important.

Broad-sense heritability ($h^2_{bs}$) refers to the portion of the phenotypic variation attributed to the overall genotypic variance and in this study, all $h^2_{bs}$ estimates were classified as very high (larger than 0.80) (Rosmaina *et al*., 2016). Narrow-sense heritability ($h^2_{ns}$) refers to the fixable portion of the phenotypic variation (Ceyhan *et al.*, 2008) and relates to additive type gene action or complementary epistatic gene interaction (Fellahi *et al*., 2013). Of the calculated $h^2_{ns}$, CHL, LW, PL, AFM, Fruit a* and IBD were estimated to be high, indicating a significant portion of this variation can be fixed in early generations. Uniformity and yield had moderately high (0.60 to 0.79) $h^2_{ns}$, and although most of the variation can be fixed in the parental lines, there is still a significant amount of variation that could be attributed to non-additive gene action. Low (less than 0.40) $h^2_{ns}$ was calculated for DMY, suggesting it is mostly under non-additive gene action, which can be exploited through hybrid breeding.

The *per se* evaluation of breeding lines can get rid of undesirable genotypes, but will not be sufficient for identifying suitable parent combinations for hybrid breeding (Fellahi *et al*., 2013). Well-performing lines can be further assessed through the calculation of GCA for relevant attributes. The top ranking lines, based on GCA effects, could be used in hybrid combinations suitable for commercial release as well as constructing a base population for breeding purposes. The reason is that these lines will have high potential in terms of transferring these characteristics to their progenies (Abrha *et al.*, 2013). High GCA estimates suggest higher heritability with less environmental effects, which will result in higher achievement through selection (Fasahat *et al*., 2016).

The testers used in this study are justified by the results presented previously. The testers meet the requirements due to superior *per se* performance, were easy to use, resulted in significant differences between crosses, and hopefully, they will reveal maximum information about the lines (Fasahat *et al*., 2016). Significant differences occurred among the lines and testers; therefore, it can be concluded that the parents had variable potential with regard to the traits evaluated (Abrha *et al*., 2013). Since a specific fruit size is an important requirement for a specific market segment, a negative AFM GCA effect can be both an advantage or detrimental, depending on the breeding objective. In cases where the requirement is a smaller fruit, G8 could be beneficial for most of the yield and other morpho-agronomic characteristics. This makes it a good general combiner but will definitely have a negative effect on internal fruit quality. Using G6 will also result in smaller fruit, but will increase DMY and FN in the hybrids without significantly reducing yield and uniformity. The use of G6 also resulted in high GCA estimates across all internal fruit quality characteristics, making it a better general combiner than G8. A good general combiner for internal fruit quality characteristics would be

G7, but it has a highly significant negative GCA effect for yield; thereby reducing its desirability. An exclusive use for G5 would be instances where large fruit sizes are particularly needed. Both lines G1 and G2 produced six positively significant GCA effects for six different traits, but unfortunately, both had a negative effect on uniformity, which is less desirable for commercial hybrids. In general, G4 was not an overall good combiner and it had a significantly negative GCA effect for yield. Initially, G3 did not stand out as a good general combiner but produced only significantly negative effects for AFM, which had been shown to be acceptable. For all other traits, GCA effects of G3 were either negligible or significantly positive, making G3 an acceptable general combiner.

Fortunately, different desirable characteristics from different parents can be combined in a single genotype through hybridisation. Therefore, G15 should be one of the top performers based on GCA alone and this is reflected in findings discussed in Chapter 3. The hybrids obtained in this way can successfully be utilised as commercial $F_1$ hybrids in order to exploit heterosis, or can be advanced to further generations for the selection of exceptional segregants for the development of outstanding recombinants after attaining homozygosity. For superior recombinants, parents with good GCA effects should be crossed with one another. Should a hybrid not show significant SCA effects, selection could be done in early segregating generations, without the fear of their true performance being masked by SCA effects (Dubey *et al*., 2014). Crosses with significant SCA are also important for the identification of homozygous parental line combinations, which could result in transgressive segregants in successive generations (Fellahi *et al*., 2013; Istipliler *et al*., 2015; Kose, 2017). In both cases, desirable genotypes with favourable genes, derived from both original parents, could be selected.

Similarly to rating parental lines on a *per se* basis, as well as through estimation of GCA effects, hybrids can be rated on a *per se* performance, and also on the basis of SCA effects, which indicate the presence of dominance and epistatic (non-additive) gene action (Dubey *et al*., 2014). The SCA effects on their own can be considered as a suitable index for determining the usefulness of a cross compared to open-pollinated varieties (Ceyhan *et al*., 2008). Highly significant SCA effects indicate that a specific cross deviates from that what would have been predicted based on the parental performances. In this study, less than 7% of the estimated SCA effects were significant. This suggested that for this population, GCA effects could predominantly be used for accurate hybrid performance prediction. This is in contrast with studies where both GCA and SCA effects were much more frequent for AFM, FN and yield (El-Tahawey *et al*., 2015; Hussein and Hamed, 2015; Ahmed *et al.*, 2017; Mohsin *et al*., 2017).

Literature suggests that the best crosses in terms of SCA effects usually involve either one or both high general combiners as parents (Shams *et al*., 2010; El-Tahawey *et al*., 2015). This was not evident in this study where the highest SCA effect for yield (G16) was a combination of G6 and G4, both of which had negative GCA effects. This is in agreement with Mohsin *et al*. (2017) who stated that good general combiners do not always produce good specific crosses with other parents, and that good specific crosses can be produced from poor general combiners.

Different parents, observed to be good general combiners for separate characteristics, may not only complement one another for the improvement of a specific characteristic, but can also complement one another by combining different desirable traits within the same genotype. This is in addition to the added advantage of heterotic yield (Hussein and Hamed, 2015)

Heterosis serves as a third method of evaluating hybrids. It is an important genetic parameter of which, depending on the characteristic and the objective of the programme, both positive and negative values could be beneficial (Kose, 2017). SCA effects can be viewed as a measure of heterosis (Ahmed *et al*., 2017). In this study, negative heterosis would only be beneficial for Leaf a*, where a negative association exists with AFM, DMY and yield; and for LW, which had a negative association with FN and yield in specific environments. Results showed significant heterosis were present for all characteristics, even though for some, the estimates were close to zero. The average heterosis for plant characteristics was equal to or below 10 and is, therefore, of relatively low commercial value. With the exception of uniformity, the yield component characteristics displayed much larger heterotic effects, which is in agreement with the high portion of the genotypic variation attributed to PvsC component in the ANOVA. Undoubtedly, any heterosis for these traits would be of benefit to the commercial grower. However, for AFM, which is a market segment defining characteristic, breeders should be aware that heterosis could result in a hybrid being suited to a different market segment when compared to that of the parental lines.

In this study, maximum heterosis was recorded for AFM, FN and yield and was 45%, 46% and 107%, respectively, which is much lower than the 82%, 88% and 171% as recorded by Ahmed *et al*. (2017). However, the yield heterosis in this study was higher than the maximum heterosis recorded for yield in other *C. moschata* studies (Jahan *et al.*, 2012; El-Tahawey *et al.*, 2015; Hussein and Hamed, 2015). These studies also referred to negative heterosis for FN, AFM and yield, which was not recorded in this study. Ahmed *et al.* (2017) and Jahan *et al.* (2012) recorded TSS heterosis of -17% to 17% and -58% to -5%, which, although a wider range than in this study, remains in agreement since it is mostly negative. The negative significant

heterosis observed in TSS, DMC and Fruit a* is unfortunate, but can be explained by negative associations with AFM, FN and yield (Chapter 5), which all showed significant positive heterosis.

Highly significant correlations between the $F_1$ performance and the sum of the parental GCA effects in this study suggests that $F_1$ hybrid performance can be accurately predicted by GCA effects alone in this population. The DMY was the only characteristic with a slightly lower correlation and was the only characteristic where $F_1$ performance had a significant association with the SCA effects. This is in agreement with results from the ANOVA where 54% of the genotypic variation was attributed to the PvsC variance component and 43% crosses variation attributed to line x tester interaction variance. This is also supported by a very high degree of dominance, suggesting extreme importance of dominance variation and hybridisation.

Unfortunately, no single plant characteristic can be bred in isolation. Characteristics associate with one another differently and act diversely in breeding programmes. It is therefore important to have specific approaches for specific characteristics. Although plant morphological characteristics are of low economic importance, Chapter 5 illustrated a strong association between CHL and various other traits including AFM, FN and Fruit a*. For CHL significant heterotic effects were demonstrated with a significant PvsC variation component, which was confirmed by significant heterosis in 75% of the crosses. An average heterosis of 7.7% was estimated, which is in agreement with the average heterosis of 10.5% for CHL recorded in tropical hybrid maize (Betran *et al*., 2003). Significant line, tester and line x tester effects confirm that both additive and non-additive gene action are involved. The $\sigma^2_{GCA}/\sigma^2_{SCA}$, which was relatively close to one (0.80), indicated the importance of both additive and non-additive genetic control. With $(\sigma^2_D/\sigma^2_A)^{1/2}$ being smaller than one (0.56), the preponderance of additive gene action was indicated, and supported by $h^2_{ns}$ (0.86) and $h^2_{bs}$ (0.94) being very high. This is in agreement with a study on sunflower where $h^2_{ns}$ was recorded as 0.87 and $h^2_{bs}$ as 0.91 (Pourmohammad *et al*., 2014). Therefore, selection in early generations appears to be best suited for the improvement of CHL.

Results for LW and PL indicate a similar pattern. The predictability ratio was higher than one and the degree of dominance smaller than one, which indicated the preponderance of additive gene action. Both characteristics also demonstrated very high $h^2_{ns}$ and $h^2_{bs}$ estimates, suggesting that additive gene combinations could be fixed in early generations through recurrent selection.

Both Leaf a* and Leaf b* showed non-significant effects for PvsC, as well as for line x tester interaction. Any breeder should be cautious making assumptions based on estimations calculated from non-significant values, and further investigations would be necessary for clarity with regard to these traits. Since significant heterosis calculated for Leaf a* and Leaf b* was less than 10%, heterosis breeding will not have a large economic impact.

Significant PvsC effects for all yield and yield component characteristics indicate the presence of heterosis for all these traits. This was confirmed by significant heterosis estimates calculated for the individual crosses. Both yield and DMY showed significant heterosis for all individual hybrids, which agrees with the high percentage of genotypic variation attributed to the PvsC component. DMY had a $\sigma^2_{GCA}/\sigma^2_{SCA}$ of 0.05 and a degree of dominance in excess of two. This suggests that DMY was under non-additive genetic control with a large dominance effect and this was confirmed by a large difference between $h^2_{bs}$ and $h^2_{ns}$. Thus, hybridisation would be the most effective approach for improving DMY. Both yield and uniformity had low predictability ratios, suggesting the domination of non-additive gene action, with the degree of dominance close to unity. This implies that both additive and dominance variances are important, which is in agreement with the very high $h^2_{bs}$ and the moderately high $h^2_{ns}$. This suggests that progress could be made through selection in the early generations, but also through hybridisation, especially in the case of yield where significant heterosis was calculated as reaching over 100%.

Various other studies have referred to significant heterosis for yield in *C. moschata* ranging from 50% (Nisha and Veeraragavathatham, 2014) to above 150% (Ahmed *et al.*, 2017; Darrudi *et al.*, 2018). All were in agreement that $\sigma^2_{GCA}$ for AFM, FN and yield was larger than $\sigma^2_{SCA}$, and therefore, these traits could be improved through recurrent selection. In contrast to these reports and in agreement with this study, Jha *et al*. (2009) and Kakamari and Jagadeesha (2017) reported the predictability ratio to be smaller than one and recommended that yield should be improved through hybridisation. Abdein *et al.* (2017) and Kakamari and Jagadeesha (2017) observed very high $h^2_{bs}$ and very low $h^2_{ns}$ respectively for AFM, FN and yield. In this study, the predictability ratio was very high and the degree of dominance very low, which suggested that recurrent selection should be an appropriate tool for the improvement of AFM. This was supported by both $h^2_{bs}$ and $h^2_{ns}$ estimates approaching one. Significant heterosis for FN was calculated for various specific crosses and in some cases, it was as high as 46%. Although genetic parameters and heritability could not be calculated, it seems as if hybridisation would have a positive influence on the increase of FN in butternut.

It should be kept in mind that genetic parameters are based on a specific population. The dissimilarity in the results described by various researchers can possibly be attributed to differences in genetic material used, as well as environmental influence and G x E interactions (Istipliler *et al.*, 2015). Most research findings referred to made use of a single location in a specific season, whereas this study was considerably more comprehensive, making use of multiple environments.

All internal fruit quality characteristics yielded significant PvsC effects, indicating significant hybrid vigour as well as significant effects for both lines and testers, which suggested additive gene action. Of these characteristics, only Fruit a* and IBD showed significant line x tester effects, implying non-additive gene action. For internal fruit quality characteristics, the predictability ratios for Fruit a* and IBD were larger than one, with the degree of dominance being smaller than one. This suggests that these characteristics were mostly under additive gene action, with limited influence from dominance variance. The $h_{bs}^2$ and $h_{ns}^2$ for both traits were very high. Recurrent selection to fix additive gene combinations in early generations will be the most effective improvement strategy, while some low heterosis can be expected through hybridisation.

Although dominance variance could not be calculated for TSS, DMC and PEN, heterosis effects were indicated by significant PvsC effects. This was supported by significant estimates of heterosis for 13 of the 16 individual crosses in the case of TSS and DMC, and 11 of the 16 crosses in the case of PEN. The TSS, DMC and Fruit a* characteristics demonstrated mostly negative heterosis and all three were negatively associated with yield. The highly significant positive heterosis in yield could possibly explain the significant negative heterosis in internal quality. Literature has reported $\sigma_{GCA}^2$ to be larger than $\sigma_{SCA}^2$ for TSS, suggesting the domination of additive gene action (Ahmed *et al*., 2017; Mohsin *et al*., 2017; Marxmathi *et al*., 2018). Literature has also reported negative heterosis for TSS (Jahan *et al*., 2012; Ahmed *et al*., 2017).

Due to wide genotypic variation and various types of genetic control, it seems that both selection and heterosis breeding could be implemented for the improvement of a number of characteristics in butternut. More specifically CHL, LW, PL, AFM, Fruit a* and IBD are predominantly under additive genetic control and could be improved through recurrent selection in early segregating generations. For those characteristics, with the exception of Fruit a*, the top *per se* line performer was also the line with the most beneficial GCA effect. This observation agree with literature stating that, in the case of predominant additive

genotypic variance, parents can be selected on either GCA effects or on *per se* means (Darrudi *et al*., 2018).

Additive gene action can be exploited through new hybrid combinations between individuals with the highest GCA effects. From these new crosses, hybrids with the highest SCA effects can then be selected to exploit non-additive gene action for the commercialisation of new hybrids. In turn, these new hybrids can then be advanced to segregating populations where selection for recombined additive genes can be done in early generations. The exploitation of additive gene effects takes place by accumulating favourable alleles through recombination and selection (Hussein and Hamed, 2015) and gives little scope for heterosis breeding (Jha *et al.*, 2009).

Improvement through recurrent selection permits small gains from each selection cycle for significant long-term improvements (Gwanama *et al*., 2001). In contrast, DMY, uniformity and yield are mostly under non-additive gene action, which can be exploited through hybridisation, and selection should be delayed until advanced generations. Broad-sense heritability estimates for these traits were very high, while $h_{ns}^2$ for uniformity and yield were moderately high and for DMY low. According to literature, characteristics under both additive and non-additive genetic control could be improved more effectively through reciprocal recurrent selection. This approach allows the exploitation of both GCA and SCA. The scheme involves two heterozygous populations, each to be used as a tester for the other. Individual plants from populations A and B are selfed and crossed with plants from the reciprocal tester populations B and A respectively. Selections are then based on the average performance of the hybrids. This method makes use of both GCA (additive) and SCA (non-additive) effects simultaneously (Mohammed, 2009; Acquaah, 2012). Based on this study, CHL, uniformity and yield would qualify for this approach. However, the intensity of such an approach and the limited resources generally available would restrict this approach to yield only.

### 6.6 Conclusions

This study confirmed that the germplasm under evaluation has the potential for improvement of various morpho-agronomic and internal fruit quality characteristics in butternut. Additive gene action was involved in the control of all characteristics, but non-additive gene action also played a significant part in uniformity and yield, with a majority of non-additive gene action in DMY. The data also supported evidence for the involvement of significant heterosis in all traits evaluated. The lines and testers demonstrated significant GCA effects for all characteristics, with only a limited amount of crosses showing significant SCA effects. Therefore, in this population, hybrid performance can be predicted predominantly using GCA effects. Based on

the heritability estimations and differences between $h^2_{bs}$ and $h^2_{ns}$, it is recommended that most characteristics can be improved through recurrent selection in early generations for the exploitation of GCA effects. In the case of yield, uniformity and specifically DMY, more emphasis should be given to heterosis breeding as the most suitable approach for maximum genetic gain.

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# CHAPTER 7

## GENERAL CONCLUSIONS AND RECOMMENDATIONS

All 15 morpho-agronomic and internal fruit quality characteristics studied demonstrated high phenotypic variance, which permits improvement of butternut through phenotypic selection. All characteristics measured showed genotype x environment (G x E) interactions through differential ranking of the genotypes across locations and seasons. The South African standard commercial varieties were found to be suitable choices for commercial growers, although G9, G24 and G15 achieved high levels of internal fruit quality, without sacrificing yield. The G x E interactions were successfully visualised for a number of yield dependant and quality characteristics using additive main effects and multiplicative interaction as well as genotype main effect plus genotype x environment interaction (GGE) biplots. The most desirable genotypes revealed high yields, desirable quality performance as well as high stability for the various characteristics, and they were different from those selected based on the combined analysis of variance across locations and seasons. These desirable genotypes included G11 for the processing segment, G16 and G17 for the fresh market segment and G13 for the small-fruited market segment, and they did not include any of the standard commercial varieties. Since the best performance was not necessarily associated with the most stable genotypes, there remain opportunities to develop high-performing varieties, with stable performance across a wide range of environmental conditions. Further investigation should be considered to generate breeding populations from the most desirable hybrids to select for high performance in early generations, but more specifically for stability in advanced generations.

In addition to phenotypic variance, high levels of genotypic variance in all characteristics indicated inherent variability, which can be utilised for the improvement of butternut. The estimation of the phenotypic coefficients of variance (PCV) demonstrated higher levels of variation for yield and yield component characteristics compared to the variation for plant and internal fruit quality characteristics. The findings demonstrated genotypic coefficients of variance (GCV) for plant characteristics to be lower compared to all other traits in this study, suggesting their improvement through selection to be more challenging. Average fruit mass (AFM) and fruit number (FN) demonstrated a narrow range of differences between PCV and GCV, indicating these traits to be governed predominantly by genetic factors with limited environmental influence on the phenotype. Therefore, selection based on phenotypic values could be effective. Results demonstrated AFM, FN, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in the fruit mesocarp (Fruit a*) and penetrometer readings as an indication of mesocarp firmness (PEN) to have high broad-sense heritability

estimates, accompanied by high genetic gain as a percentage of the population mean (GAM). As a result, selection in early generations would be effective for improvement of these traits. The green-red colour contribution in the leaves, dry matter yield (DMY), uniformity and yield showed low heritability and low GAM, implying progress through simple selection would be ineffective. Characteristics with the highest GCV and GAM have the largest scope for improvement in butternut. These characteristics include AFM and FN as yield-dependent traits, and Fruit a* and TSS as quality traits. All four these characteristics could be improved through selection in early generations.

The genotypic and phenotypic correlations suggested leaf chlorophyll content (CHL) to have moderate associations with AFM and FN. However, these associations would be difficult to utilise through indirect selection due to relatively low GCV in CHL. The introduction of material to increase the genetic variation in plant characteristics could have significant benefits. Results further indicated yield to have a weak negative genotypic association with quality characteristics including TSS, DMC and Fruit a*. Various internal quality characteristics had weak positive genotypic associations among one another of which the strongest was between DMC and TSS. Since it is easier and faster to work with TSS, it is the characteristic of choice. Although PEN had a weak association with TSS and DMC, it is still of value where large numbers of fruit need to be assessed in early segregating populations. The greatest benefit of working with PEN is instant results during evaluation.

Path coefficient analysis indicated FN and to a lesser extent AFM, internal breakdown and leaf width to have recurring direct effects on yield, which should be considered during the improvement of yield in butternut. However, the strong negative association between FN and AFM should be taken note of. The reason is that an increase in FN could result in higher yield but, at the same time, may result in a significant reduction in AFM. This will consequently disqualify a variety from a specific market segment. A complex characteristic such as yield should not be considered on its own, especially for simultaneous improvement of internal fruit quality.

Additive gene action was involved in the control of all measured characteristics. Non-additive gene action also played a significant part in uniformity and yield, with a majority of non-additive gene action involved in DMY. Results further supported evidence for the involvement of significant heterosis in all traits evaluated. The lines and testers used in crosses demonstrated significant general combining ability (GCA) effects for all characteristics. Only a limited number of crosses demonstrated significant specific combining ability effects, which suggested that hybrid performance could be predicted predominantly using GCA effects. For the exploitation

of GCA effects through additive genetic control, the possibility to improve FN and yield should be investigated in segregating populations developed from G13 (BUT01xBUT06) and G21 (BUT1xBUT8). Similarly, superior internal fruit quality should be investigated in segregating populations developed from G15 (BUT03xBUT06) and G19 (BUT03xBUT07). Additional crosses for improvement of internal fruit quality should be considered between BUT06 and BUT07.

Based on the heritability estimations and differences between broad-sense and narrow-sense heritability, it is recommended that most characteristics could be improved through recurrent selection in early generations for the exploitation of GCA effects. In the case of yield, uniformity and specifically DMY, more emphasis should be given to heterosis breeding as the most suitable approach for maximum genetic gain. Since higher heterosis was estimated for yield than for AFM and FN, it should be considered to improve FN through selection in early generations and consider non-additive genetic control in yield as a hybridisation effect. However, the heterosis effect in AFM should be taken into account when crosses are designed for a specific market segment.

Biofortification as a component of vegetable breeding is a promising strategy to increase carotenoid concentration in agricultural products. The health benefits associated with the prevention of vitamin A deficiency could further increase the popularity of butternut. For future research, carotenoid profiling should be included in a similar study. There is a need to investigate specific and total carotenoid content associated with other internal fruit quality characteristics. This study can also be expanded to include correlations between carotenoids and yield component characteristics.

## Appendix

**Table A1** Estimates for genetic parameters for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform) and yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) Jacobsdal 2018/2019 (E1)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Max | 52.10 | -13.87 | 34.40 | 365.00 | 530.00 | 3.61 | 19.79 | 101.00 | 80.00 | 170.12 | 10.69 | 16.25 | 25.87 | 90.00 | 4.03 |
| Min | 26.96 | -22.88 | 21.45 | 255.00 | 279.00 | 1.00 | 8.49 | 28.00 | 40.00 | 77.70 | 5.35 | 6.91 | 4.19 | 36.00 | 1.82 |
| Mean | 39.91 | -19.36 | 27.74 | 322.34 | 400.36 | 2.17 | 12.62 | 59.30 | 57.28 | 118.30 | 7.65 | 10.85 | 18.01 | 78.29 | 2.97 |
| SEM | 1.70 | 0.84 | 0.98 | 7.65 | 13.84 | 0.13 | 1.36 | 6.22 | 5.00 | 11.55 | 0.56 | 0.81 | 1.46 | 4.34 | 0.12 |
| CD 5% | 4.82 | 2.39 | 2.77 | 21.70 | 39.27 | 0.37 | 3.85 | 17.64 | 14.19 | 32.78 | 1.59 | 2.29 | 4.14 | 12.31 | 0.34 |
| $\sigma_e^2$ | 8.64 | 2.12 | 2.86 | 175.34 | 574.46 | 0.05 | 5.51 | 115.98 | 75.02 | 400.37 | 0.94 | 1.95 | 6.38 | 56.42 | 0.04 |
| $\sigma_g^2$ | 14.60 | 2.06 | 4.71 | 327.76 | 1457.24 | 0.48 | -0.27 | 215.39 | 70.75 | 109.71 | 0.65 | 1.34 | 8.36 | 143.62 | 0.09 |
| $\sigma_p^2$ | 23.25 | 4.18 | 7.56 | 503.10 | 2031.70 | 0.53 | 5.24 | 331.37 | 145.77 | 510.09 | 1.59 | 3.29 | 14.74 | 200.04 | 0.13 |
| ECV | 7.37 | -7.53 | 6.10 | 4.11 | 5.99 | 10.32 | 18.61 | 18.16 | 15.12 | 16.91 | 12.68 | 12.86 | 14.03 | 9.59 | 7.00 |
| GCV | 9.57 | -7.41 | 7.82 | 5.62 | 9.53 | 32.05 | 4.10 | 24.75 | 14.68 | 8.85 | 10.53 | 10.68 | 16.05 | 15.31 | 10.06 |
| PCV | 12.08 | -10.56 | 9.92 | 6.96 | 11.26 | 33.67 | 18.15 | 30.70 | 21.08 | 19.09 | 16.48 | 16.72 | 21.32 | 18.07 | 12.26 |
| $h_{bs}^2$ | 0.63 | 0.49 | 0.62 | 0.65 | 0.72 | 0.91 | | 0.65 | 0.49 | 0.22 | 0.41 | 0.41 | 0.57 | 0.72 | 0.67 |
| GA | 6.24 | 2.07 | 3.52 | 30.10 | 66.60 | 1.36 | | 24.37 | 12.07 | 10.01 | 1.06 | 1.53 | 4.48 | 20.92 | 0.51 |
| GAM | 15.63 | -10.71 | 12.71 | 9.34 | 16.63 | 62.84 | | 41.11 | 21.07 | 8.46 | 13.87 | 14.06 | 24.90 | 26.72 | 17.02 |

Max: Maximum, Min: Minimum, SEM: Standard error of mean, CD: Critical difference, $\sigma_e^2$: Environmental variance, $\sigma_g^2$: Genotypic variance, $\sigma_p^2$: Phenotypic variance, ECV: Environmental coefficient of variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, $h_{bs}^2$: Broad-sense heritability, GA: Genetic advance, GAM: Genetic advance as percentage of mean

**Table A2** Estimates for genetic parameters for across six environments for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform) and yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) Jacobsdal 2019/2020 (E2)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Max | 60.69 | -15.32 | 33.70 | 348.00 | 428.00 | 3.91 | 6.77 | 50.00 | 80.00 | 78.46 | 10.88 | 13.84 | 24.84 | 90.00 | 3.29 |
| Min | 35.12 | -25.69 | 19.58 | 253.00 | 292.00 | 1.07 | 1.90 | 9.00 | 40.00 | 19.26 | 4.75 | 6.02 | 3.86 | 20.00 | 1.46 |
| Mean | 45.59 | -21.23 | 26.91 | 307.56 | 364.16 | 1.98 | 4.46 | 26.88 | 59.14 | 48.96 | 7.26 | 9.18 | 15.67 | 76.04 | 2.50 |
| SEM | 1.63 | 0.80 | 0.79 | 6.90 | 10.19 | 0.10 | 0.30 | 2.06 | 4.16 | 3.66 | 0.40 | 0.45 | 1.41 | 4.42 | 0.10 |
| CD 5% | 4.62 | 2.28 | 2.25 | 19.57 | 28.92 | 0.28 | 0.86 | 5.86 | 11.79 | 10.40 | 1.13 | 1.27 | 4.01 | 12.54 | 0.27 |
| $\sigma_e^2$ | 7.94 | 1.94 | 1.88 | 142.63 | 311.47 | 0.03 | 0.27 | 12.78 | 51.80 | 40.28 | 0.48 | 0.60 | 5.99 | 58.56 | 0.03 |
| $\sigma_g^2$ | 26.77 | 2.98 | 5.11 | 197.55 | 415.42 | 0.33 | 1.33 | 91.94 | 47.34 | 141.30 | 1.31 | 2.08 | 12.62 | 137.66 | 0.10 |
| $\sigma_p^2$ | 34.71 | 4.92 | 6.99 | 340.18 | 726.89 | 0.36 | 1.60 | 104.72 | 99.15 | 181.59 | 1.79 | 2.68 | 18.61 | 196.22 | 0.13 |
| ECV | 6.18 | -6.57 | 5.10 | 3.88 | 4.85 | 8.80 | 11.71 | 13.30 | 12.17 | 12.96 | 9.52 | 8.43 | 15.62 | 10.06 | 6.69 |
| GCV | 11.35 | -8.13 | 8.40 | 4.57 | 5.60 | 29.21 | 25.84 | 35.68 | 11.64 | 24.28 | 15.79 | 15.69 | 22.66 | 15.43 | 12.71 |
| PCV | 12.92 | -10.45 | 9.82 | 6.00 | 7.40 | 30.51 | 28.37 | 38.07 | 16.84 | 27.52 | 18.44 | 17.82 | 27.52 | 18.42 | 14.37 |
| $h_{bs}^2$ | 0.77 | 0.61 | 0.73 | 0.58 | 0.57 | 0.92 | 0.83 | 0.88 | 0.48 | 0.78 | 0.73 | 0.78 | 0.68 | 0.70 | 0.78 |
| GA | 9.36 | 2.76 | 3.98 | 22.06 | 31.74 | 1.14 | 2.16 | 18.51 | 9.79 | 21.60 | 2.02 | 2.62 | 6.02 | 20.24 | 0.58 |
| GAM | 20.54 | -13.02 | 14.79 | 7.17 | 8.72 | 57.63 | 48.48 | 68.86 | 16.56 | 44.12 | 27.85 | 28.48 | 38.44 | 26.62 | 23.17 |

Max: Maximum, Min: Minimum, SEM: Standard error of mean, CD: Critical difference, $\sigma_e^2$: Environmental variance, $\sigma_g^2$: Genotypic variance, $\sigma_p^2$: Phenotypic variance, ECV: Environmental coefficient of variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, $h_{bs}^2$: Broad-sense heritability, GA: Genetic advance, GAM: Genetic advance as percentage of mean

**Table A3** Estimates for genetic parameters for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform) and yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) for Kaalfontein 2018/2019 (E3)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Max | 48.04 | -16.10 | 34.35 | 299.00 | 415.00 | 1.90 | 6.22 | 76.00 | 80.00 | 66.02 | 11.03 | 13.08 | 23.26 | 90.00 | 4.07 |
| Min | 27.68 | -23.21 | 24.48 | 228.00 | 246.00 | 0.57 | 2.98 | 25.00 | 40.00 | 32.20 | 4.90 | 6.37 | 6.31 | 50.00 | 1.88 |
| Mean | 36.26 | -20.15 | 30.26 | 265.10 | 305.60 | 1.08 | 4.66 | 48.11 | 61.11 | 48.61 | 7.77 | 9.73 | 17.50 | 79.19 | 3.05 |
| SEM | 1.33 | 0.40 | 0.63 | 4.19 | 10.01 | 0.08 | 0.34 | 3.36 | 4.98 | 2.29 | 0.49 | 0.57 | 1.05 | 5.04 | 0.15 |
| CD 5% | 3.79 | 1.13 | 1.79 | 11.89 | 28.41 | 0.23 | 0.96 | 9.52 | 14.13 | 6.50 | 1.40 | 1.61 | 2.98 | 14.31 | 0.42 |
| $\sigma_e^2$ | 5.34 | 0.48 | 1.20 | 52.66 | 300.67 | 0.02 | 0.34 | 33.79 | 74.36 | 15.75 | 0.73 | 0.96 | 3.31 | 76.33 | 0.07 |
| $\sigma_g^2$ | 7.21 | 2.77 | 4.78 | 167.36 | 992.06 | 0.09 | 0.18 | 127.68 | 42.74 | 47.05 | 1.25 | 1.72 | 11.75 | 59.69 | 0.11 |
| $\sigma_p^2$ | 12.55 | 3.24 | 5.97 | 220.02 | 1292.73 | 0.11 | 0.52 | 161.47 | 117.09 | 62.80 | 1.98 | 2.68 | 15.07 | 136.02 | 0.18 |
| ECV | 6.37 | -3.42 | 3.62 | 2.74 | 5.67 | 13.22 | 12.53 | 12.08 | 14.11 | 8.16 | 10.98 | 10.07 | 10.40 | 11.03 | 8.37 |
| GCV | 7.40 | -8.26 | 7.22 | 4.88 | 10.31 | 28.31 | 9.21 | 23.49 | 10.70 | 14.11 | 14.39 | 13.47 | 19.59 | 9.76 | 10.88 |
| PCV | 9.77 | -8.94 | 8.08 | 5.60 | 11.77 | 31.25 | 15.55 | 26.41 | 17.71 | 16.30 | 18.10 | 16.82 | 22.19 | 14.73 | 13.73 |
| $h_{bs}^2$ | 0.57 | 0.85 | 0.80 | 0.76 | 0.77 | 0.82 | 0.35 | 0.79 | 0.37 | 0.75 | 0.63 | 0.64 | 0.78 | 0.44 | 0.63 |
| GA | 4.19 | 3.17 | 4.03 | 23.24 | 56.84 | 0.57 | 0.52 | 20.70 | 8.14 | 12.23 | 1.83 | 2.16 | 6.24 | 10.54 | 0.54 |
| GAM | 11.56 | -15.71 | 13.30 | 8.77 | 18.60 | 52.85 | 11.24 | 43.02 | 13.31 | 25.16 | 23.56 | 22.22 | 35.65 | 13.31 | 17.77 |

Max: Maximum, Min: Minimum, SEM: Standard error of mean, CD: Critical difference, $\sigma_e^2$: Environmental variance, $\sigma_g^2$: Genotypic variance, $\sigma_p^2$: Phenotypic variance, ECV: Environmental coefficient of variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, $h_{bs}^2$: Broad-sense heritability, GA: Genetic advance, GAM: Genetic advance as percentage of mean

**Table A4** Estimates for genetic parameters for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform) and yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) for Kaalfontein 2019/2020 (E4)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Max | 43.44 | -19.86 | 35.59 | 336.00 | 561.00 | 3.39 | 9.88 | 120.00 | 70.00 | 122.28 | 9.65 | 12.83 | 26.02 | 90.00 | 3.41 |
| Min | 27.10 | -29.43 | 23.05 | 246.00 | 308.00 | 0.79 | 5.30 | 26.00 | 30.00 | 49.62 | 4.67 | 6.79 | 8.17 | 60.00 | 1.82 |
| Mean | 35.25 | -25.33 | 29.67 | 292.56 | 385.33 | 1.36 | 7.42 | 63.63 | 49.63 | 80.59 | 6.89 | 9.35 | 18.13 | 88.10 | 2.62 |
| SEM | 1.11 | 0.95 | 1.24 | 5.62 | 10.29 | 0.08 | 0.52 | 3.99 | 4.30 | 4.77 | 0.30 | 0.37 | 1.04 | 2.68 | 0.09 |
| CD 5% | 3.14 | 2.69 | 3.51 | 15.95 | 29.19 | 0.22 | 1.46 | 11.33 | 12.20 | 13.53 | 0.86 | 1.04 | 2.95 | 7.62 | 0.25 |
| $\sigma_e^2$ | 3.66 | 2.69 | 4.60 | 94.80 | 317.40 | 0.02 | 0.80 | 47.79 | 55.41 | 68.15 | 0.27 | 0.40 | 3.25 | 21.61 | 0.02 |
| $\sigma_g^2$ | 9.12 | 1.60 | 4.60 | 110.99 | 1577.54 | 0.19 | 0.25 | 285.81 | 32.24 | 127.35 | 1.27 | 1.56 | 7.96 | 0.00 | 0.09 |
| $\sigma_p^2$ | 12.78 | 4.28 | 9.19 | 205.79 | 1894.94 | 0.21 | 1.05 | 333.60 | 87.65 | 195.50 | 1.54 | 1.96 | 11.21 | 21.61 | 0.12 |
| ECV | 5.43 | -6.47 | 7.23 | 3.33 | 4.62 | 9.75 | 12.05 | 10.86 | 15.00 | 10.24 | 7.59 | 6.79 | 9.94 | 5.28 | 5.77 |
| GCV | 8.56 | -4.99 | 7.22 | 3.60 | 10.31 | 31.93 | 6.72 | 26.57 | 11.44 | 14.00 | 16.36 | 13.34 | 15.57 | 0.00 | 11.66 |
| PCV | 10.14 | -8.17 | 10.22 | 4.90 | 11.30 | 33.39 | 13.80 | 28.70 | 18.86 | 17.35 | 18.04 | 14.97 | 18.47 | 5.28 | 13.01 |
| $h_{bs}^2$ | 0.71 | 0.37 | 0.50 | 0.54 | 0.83 | 0.91 | 0.24 | 0.86 | 0.37 | 0.65 | 0.82 | 0.79 | 0.71 | 0.00 | 0.80 |
| GA | 5.25 | 1.59 | 3.12 | 15.94 | 74.65 | 0.86 | 0.50 | 32.24 | 7.09 | 18.76 | 2.11 | 2.29 | 4.90 | 0.00 | 0.56 |
| GAM | 14.90 | -6.27 | 10.52 | 5.45 | 19.37 | 62.92 | 6.74 | 50.66 | 14.29 | 23.28 | 30.57 | 24.49 | 27.02 | 0.00 | 21.53 |

Max: Maximum, Min: Minimum, SEM: Standard error of mean, CD: Critical difference, $\sigma_e^2$: Environmental variance, $\sigma_g^2$: Genotypic variance, $\sigma_p^2$: Phenotypic variance, ECV: Environmental coefficient of variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, $h_{bs}^2$: Broad-sense heritability, GA: Genetic advance, GAM: Genetic advance as percentage of mean

**Table A5** Estimates for genetic parameters for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform) and yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) for Oudtshoorn 2018/2019 (E5)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Max | 56.07 | -9.39 | 28.19 | 370.00 | 419.00 | 4.25 | 14.41 | 98.00 | 80.00 | 141.88 | 10.44 | 13.91 | 22.20 | 90.00 | 3.28 |
| Min | 30.39 | -19.53 | 17.52 | 246.00 | 234.00 | 1.09 | 4.95 | 18.00 | 40.00 | 65.10 | 5.13 | 6.80 | 6.99 | 34.00 | 1.88 |
| Mean | 45.05 | -14.22 | 22.14 | 302.27 | 340.90 | 2.09 | 9.14 | 51.86 | 59.14 | 99.78 | 7.26 | 9.27 | 15.56 | 73.51 | 2.56 |
| SEM | 2.68 | 1.05 | 0.98 | 10.35 | 10.65 | 0.11 | 0.72 | 4.81 | 3.32 | 6.66 | 0.42 | 0.48 | 1.08 | 4.81 | 0.08 |
| CD 5% | 7.61 | 2.98 | 2.79 | 29.37 | 30.22 | 0.31 | 2.05 | 13.65 | 9.41 | 18.91 | 1.20 | 1.36 | 3.07 | 13.66 | 0.23 |
| $\sigma_e^2$ | 21.60 | 3.30 | 2.89 | 321.33 | 340.17 | 0.04 | 1.57 | 69.45 | 33.00 | 133.23 | 0.54 | 0.68 | 3.51 | 69.46 | 0.02 |
| $\sigma_g^2$ | 9.63 | 2.46 | 3.27 | 282.66 | 946.07 | 0.44 | 1.33 | 210.22 | 90.36 | 197.52 | 1.03 | 1.79 | 7.29 | 207.26 | 0.07 |
| $\sigma_p^2$ | 31.23 | 5.76 | 6.16 | 603.99 | 1286.24 | 0.47 | 2.91 | 279.67 | 123.36 | 330.75 | 1.57 | 2.47 | 10.79 | 276.73 | 0.09 |
| ECV | 10.32 | -12.77 | 7.68 | 5.93 | 5.41 | 9.10 | 13.71 | 16.07 | 9.71 | 11.57 | 10.11 | 8.92 | 12.03 | 11.34 | 5.36 |
| GCV | 6.89 | -11.04 | 8.17 | 5.56 | 9.02 | 31.55 | 12.64 | 27.96 | 16.07 | 14.09 | 13.94 | 14.42 | 17.35 | 19.59 | 10.16 |
| PCV | 12.40 | -16.88 | 11.21 | 8.13 | 10.52 | 32.84 | 18.65 | 32.24 | 18.78 | 18.23 | 17.22 | 16.95 | 21.11 | 22.63 | 11.49 |
| $h_{bs}^2$ | 0.31 | 0.43 | 0.53 | 0.47 | 0.74 | 0.92 | 0.46 | 0.75 | 0.73 | 0.60 | 0.66 | 0.72 | 0.68 | 0.75 | 0.78 |
| GA | 3.55 | 2.11 | 2.71 | 23.69 | 54.34 | 1.31 | 1.61 | 25.90 | 16.76 | 22.37 | 1.69 | 2.34 | 4.57 | 25.67 | 0.47 |
| GAM | 7.88 | -14.87 | 12.25 | 7.84 | 15.94 | 62.45 | 17.64 | 49.93 | 28.34 | 22.42 | 23.26 | 25.26 | 29.37 | 34.92 | 18.50 |

Max: Maximum, Min: Minimum, SEM: Standard error of mean, CD: Critical difference, $\sigma_e^2$: Environmental variance, $\sigma_g^2$: Genotypic variance, $\sigma_p^2$: Phenotypic variance, ECV: Environmental coefficient of variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, $h_{bs}^2$: Broad-sense heritability, GA: Genetic advance, GAM: Genetic advance as percentage of mean

**Table A6** Estimates for genetic parameters (E6) for chlorophyll content (CHL), green-red colour contribution in the leaf canopy (Leaf a*), yellow-blue colour contribution in the leaf canopy (Leaf b*), leaf width (LW) and petiole length (PL), average fruit mass (AFM), dry matter yield (DMY), fruit number (FN), uniformity (Uniform) and yield, total soluble solids (TSS), dry matter content (DMC), green-red colour contribution in fruit mesocarp (Fruit a*), internal breakdown (IBD), mesocarp penetrometer reading (PEN) for Oudtshoorn 2019/2020 (E6)

| | CHL | Leaf a* | Leaf b* | LW | PL | AFM | DMY | FN | Uniform | Yield | TSS | DMC | Fruit a* | IBD | PEN |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Max | 67.13 | -9.53 | 25.26 | 362.00 | 442.00 | 4.10 | 15.26 | 89.00 | 80.00 | 138.10 | 12.65 | 14.82 | 24.79 | 90.00 | 3.31 |
| Min | 37.60 | -22.94 | 12.77 | 194.00 | 222.00 | 0.98 | 4.00 | 21.00 | 30.00 | 43.75 | 5.67 | 7.19 | 12.79 | 16.00 | 1.33 |
| Mean | 50.91 | -15.31 | 19.55 | 256.07 | 297.86 | 1.84 | 9.67 | 52.69 | 57.04 | 90.15 | 8.66 | 10.77 | 18.76 | 74.40 | 2.54 |
| SEM | 2.53 | 1.36 | 0.92 | 10.81 | 14.50 | 0.12 | 0.96 | 4.89 | 4.97 | 9.64 | 0.50 | 0.53 | 1.06 | 7.11 | 0.10 |
| CD 5% | 7.18 | 3.85 | 2.60 | 30.68 | 41.15 | 0.35 | 2.71 | 13.88 | 14.11 | 27.36 | 1.43 | 1.50 | 3.02 | 20.19 | 0.29 |
| $\sigma_e^2$ | 19.20 | 5.52 | 2.52 | 350.67 | 630.85 | 0.05 | 2.74 | 71.81 | 74.22 | 278.89 | 0.76 | 0.84 | 3.40 | 151.83 | 0.03 |
| $\sigma_g^2$ | 14.74 | 2.13 | 4.26 | 263.50 | 525.84 | 0.33 | 2.62 | 195.71 | 90.08 | 103.24 | 0.97 | 1.35 | 4.52 | 210.60 | 0.11 |
| $\sigma_p^2$ | 33.94 | 7.65 | 6.77 | 614.17 | 1156.69 | 0.38 | 5.36 | 267.52 | 164.29 | 382.13 | 1.72 | 2.19 | 7.92 | 362.43 | 0.14 |
| ECV | 8.61 | -15.34 | 8.11 | 7.31 | 8.43 | 11.75 | 17.13 | 16.08 | 15.10 | 18.52 | 10.05 | 8.49 | 9.83 | 16.56 | 6.87 |
| GCV | 7.54 | -9.53 | 10.55 | 6.34 | 7.70 | 31.40 | 16.73 | 26.55 | 16.64 | 11.27 | 11.35 | 10.81 | 11.34 | 19.51 | 12.78 |
| PCV | 11.44 | -18.06 | 13.31 | 9.68 | 11.42 | 33.53 | 23.95 | 31.04 | 22.47 | 21.68 | 15.16 | 13.74 | 15.01 | 25.59 | 14.51 |
| $h_{bs}^2$ | 0.43 | 0.28 | 0.63 | 0.43 | 0.45 | 0.88 | 0.49 | 0.73 | 0.55 | 0.27 | 0.56 | 0.62 | 0.57 | 0.58 | 0.78 |
| GA | 5.21 | 1.59 | 3.37 | 21.90 | 31.85 | 1.12 | 2.33 | 24.65 | 14.48 | 10.88 | 1.52 | 1.89 | 3.31 | 22.79 | 0.59 |
| GAM | 10.24 | -10.36 | 17.23 | 8.55 | 10.69 | 60.59 | 24.09 | 46.78 | 25.38 | 12.07 | 17.50 | 17.51 | 17.66 | 30.63 | 23.19 |

Max: Maximum, Min: Minimum, SEM: Standard error of mean, CD: Critical difference, $\sigma_e^2$: Environmental variance, $\sigma_g^2$: Genotypic variance, $\sigma_p^2$: Phenotypic variance, ECV: Environmental coefficient of variance, PCV: Phenotypic coefficient of variation, GCV: Genotypic coefficient of variation, $h_{bs}^2$: Broad-sense heritability, GA: Genetic advance, GAM: Genetic advance as percentage of mean