UNIVERSITY OF KWAZULU-NATAL

CHARACTERISATION OF TARO (*COLOCASIA ESCULENTA* (L) SCHOTT) IN SOUTH AFRICA: TOWARDS BREEDING AN ORPHAN CROP

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CHARACTERISATION OF TARO (*COLOCASIA ESCULENTA* (L) SCOTT) GERMPLASM COLLECTIONS IN SOUTH AFRICA: TOWARDS BREEDING AN ORPHAN CROP

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DECLARATION

I, Willem Jansen van Rensburg, declare that:

1. The research reported in this thesis, except where otherwise indicated, is my original work and has not been submitted for any degree or examination at any other university.
2. This thesis does not contain data, pictures, graphs or other information from other researchers, unless specifically acknowledged as being sourced from other persons.
3. This thesis does not contain other persons' writing, unless acknowledged as being sourced from other researchers. Where other written sources have been quoted, then their words have been re-written and the information attributed to them has been referenced.
4. This study was funded by the European Union and the Department of Agriculture, Forestry and Fisheries

Signed

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Dr MW Bairu (Co-Supervisor- ARC)

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Professor H Shimelis (Co-Supervisor)

DEDICATION

This thesis is dedicated to my parents
Koos and Ria Jansen van Rensburg
I would have been nothing without you

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General Abstract

Amadumbe (*Colocasia esculenta*), better known as taro, is a traditional root crop widely cultivated in the coastal areas of South Africa. Taro is showing potential for commercialisation. However, very little is known about the genetic diversity, potential and its introduction and movement in South Africa. This study was undertaken to (1) determine the genetic diversity in the ARC taro germplasm collection using agro-morphological characteristics and microsatellite markers, (2) to determine if it is possibility to breed with local taro germplasm and (3) to determine the effect of four different environments (Roodeplaat, Umbumbulu, Owen Sithole College of Agriculture and Nelspruit) on ten agro-morphological characteristics of 29 taro landraces

Taro germplasm was collected in South Africa in order to build up a representative collection. Germplasm was also imported from Nigeria and Vanuatu. The South African taro germplasm, and selected introduced germplasm, were characterised using agro-morphological descriptors and simple sequence repeat (SSR) markers. Limited variation was observed between the South African accessions when agro-morphological descriptors were used. Non-significant variations were observed for eight of the 30 agro-morphological characteristics. The 86 accessions were grouped into three clusters each containing 39, 20 and 27 accessions, respectively. The tested SSR primers revealed polymorphisms for the South African germplasm collections. Primer Uq 84 was highly polymorphic. The SSR markers grouped the accessions into five clusters with 33, 6, 5, 41 and 7 accessions in each of the clusters. All the dasheen type taro accessions were clustered together. When grown under uniform conditions, a higher level of genetic diversity in the South African germplasm was observed when molecular (SSR) analysis was performed than with morphological characterisation. No correlation was detected between the different clusters and geographic distribution, since accessions from the same locality did not always cluster together. Conversely, accessions collected at different sites were grouped together. There was also no clear correlation between the

clustering pattern based on agro-morphology and SSRs. Thus, in order to obtain a more complete characterisation, both molecular and morphological data should be used. Although the results indicated that there is more diversity present in the local germplasm than expected, the genetic base is still rather narrow, as reported in other African countries.

Fourteen distinct taro genotypes were planted as breeding parents and grown in a glasshouse. Flowering were induced with gibberellic acid (GA_3). Crosses were performed in various combinations; however, no offspring were obtained. This might be due to the triploid nature of the South African germplasm. It might be useful to pollinate diploid female parents with triploid male parents or use advanced breeding techniques, like embryo rescue or polyploidization, to obtain offspring with the South African triploid germplasm as one parent. The triploid male parents might produce balanced gametes at low percentages, which can fertilize the diploid female parents.

Twenty-nine taro accessions were planted at three localities, representing different agro-ecological zones. These localities were Umbumbulu (South of Durban - KZN), Owen Sithole College of Agricultural (OSCA, Empangeni, KZN) and ARC - Vegetable and Ornamental Plants (Roodeplaat, Pretoria). Different growth and yield related parameters were measured. The data were subjected to analysis of variance (ANOVA) and additive main effects and multiplicative interaction (AMMI) analyses. Significant GxE was observed between locality and specific lines for mean leaf length, leaf width, leaf number, plant height, number of suckers per plant, number of cormels harvested per plant, total weight of the cormels harvested per plant and corm length. No significant interaction between the genotype and the environment was observed for the canopy diameter and corm breadth. From the AMMI model, it is clear that all the interactions are significant for leaf length, leaf width, number of leaves on a single plant, plant height, number of suckers, number of cormels harvested from a single plant and weight of cormels

harvested from a single plant. The AMMI model indicated that the main effects were significant but not the interactions for canopy diameter. The AMMI model for the length and width of the corms showed that the effect of environment was highly significant. There is a strong positive correlation between the number of suckers and the number of leaves (0.908), number of cormels (0.809) and canopy diameter (0.863) as well as between the number of leaves and the canopy diameter (0.939) and between leaf width and plant height (0.816). There is not a single genotype that can be identified as “the best” genotype. This is due to the interaction between the environments and the genotypes. Amzam174 and Thandizwe43 seem to be genotypes that are often regarded as being in the top four. For the farmer, the total weight of the cormels harvested from a plant will be the most important. Thandizwe43, Mabhida and Amzam174 seem to be some of the better genotypes for the total weight and number of cormels harvested from a single plant and can be promoted under South African taro producers. The local accessions also perform better than introduced accessions. It is clear that some of the introduced accessions do have the potential to be commercialised in South Africa.

The study indicate that there are genetic diversity that can be tapped into for breeding of taro in South Africa. However, hand pollination techniques should be optimized. Superior genotypes within each cluster in the dendrograms as well as Thandizwe43, Mabhida and Amzam174 (identified by the AMMI analysis as high yielding) can be identified and used as parents in a clonal selection and breeding programme. Additionally, more diploid germplasm can be imported to widen the genetic base. The choice of germplasm must be done with caution to obtain germplasm adapted to South African climate and for acceptable for the South African consumers.

Key words: accessions, agro-ecological zones, agro-morphological characteristics, local germplasm, polymorphism and taro

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CHAPTER 1: LITERATURE REVIEW

1.1 *Colocasia esculenta* (L) Schott. (Taro, Amadumbe)

Amadumbe (*Colocasia esculenta*) is a popular starch crop in certain parts of South Africa (Modi 2007, Mabhaudhi 2012). Amadumbe is the isiZulu vernacular for taro, dasheen, eddoe, cocoyam or elephant as it is better known throughout the rest of the world (Safo Kantaka 2004, Mabhaudhi 2012). It is a popular starch staple in tropical Africa, Asia, Pacific Islands and Americas (Lebot 2009). Lebot reported that taro is still regarded as an orphan crop, commonly cultivated in home gardens or in shifting agroforestry with limited input. There are no commercial taro cultivars in South Africa and research on taro is inadequate when compared with that of conventional root and tuber crops (Modi 2007).

1.1.1 Scientific classification

Colocasia esculenta (L.) Schott

Protologue:	Schott & Endl., Melet. bot.: 18 (1832).
Family:	Araceae
Chromosome number:	2*n* = 28, 42, 56 and x = 14
Synonyms:	*Colocasia antiquorum* Schott (1832).

The genus *Colocasia* consist of eight species from tropical Asia and is classified in the tribe *Colocasieae*, along with *Alocasia*. There are two variety-groups of taro, the Dasheen Group which consists of a single large corm producing a few small cormels (Figure 1.1) and the Eddoe Group (frequently classified as *C. esculenta* var. *antiquorum* (Schott) F.T.Hubb. & Rehder) producing many cormels of varying size (Figure 1.2) (Safo Kantaka 2004; Lebot 2009). Most taro landraces in South Africa belong to the Eddoe Group.

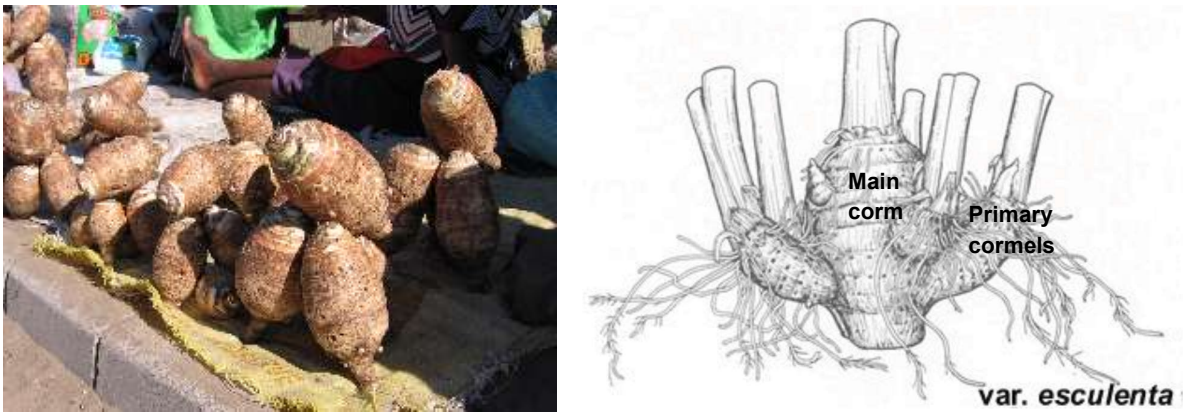

Figure 1.1: Dasheen type taro (*Colocasia esculenta* var. *esculenta*). Left: Dasheen type corms on display by informal vendors in Manguzi, KwaZulu-Natal. This landrace was not included in the study (Photo: WS Jansen van Rensburg). Right: Line drawing of the dasheen type corm and cormels. The main corm and primary cormels can be distinguished (Curtesy of the Bishop Museum, Hawaii).

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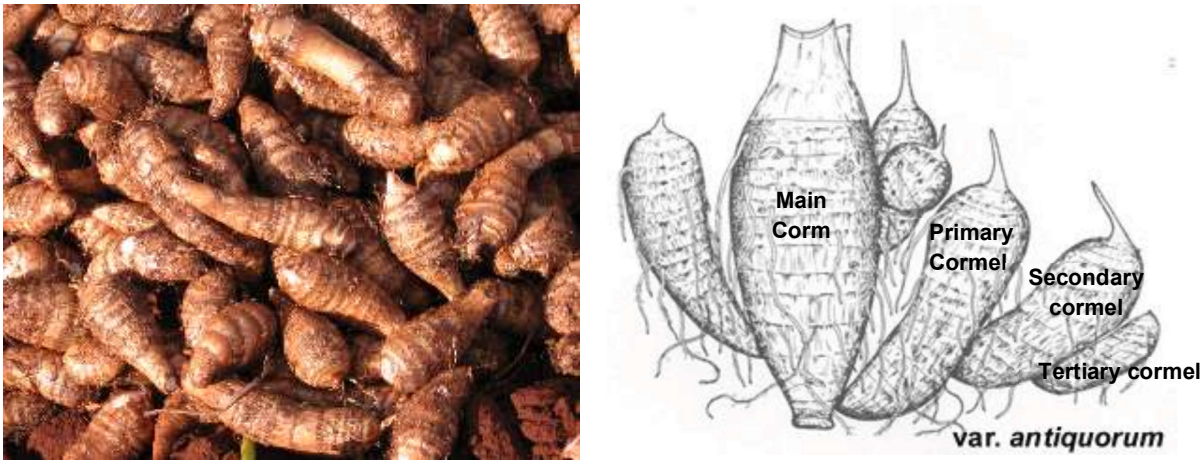

Figure 1.2: Eddoe type taro (*Colocasia esculenta* var. *antiquorun*). Left: Eddoe type corms on display by informal vendors in Manguzi, KwaZulu-Natal (Photo: WS Jansen van Rensburg). Right: Line drawing of the dasheen type corm and cormels. The main corm, primary, secondary and tertiary cormels can de distinguished clearly (Curtesy of the Bishop Museum, Hawaii).

1.1.2 Description

Taro is an erect perennial herb up to 2 metres tall, but is mostly cultivated as an annual (Safo Kantaka 2004; Lebot 2009). The root system is adventitious, fibrous and shallow.

The storage stem (corm) is usually brown and marked by a number of rings, it is cylindrical or spherical in shape and may grow to be very large - up to 4 kg (Figure 1.1 and 1.2). The lateral buds give rise to cormels, suckers or stolons. The leaves are arranged in a rosette and are simple and peltate (Figure 1.3). The petiole can be up to 1 m long, with distinct sheath. The leaf blades are cordate, up to 85 × 60 cm, entire, glabrous, with three main veins and rounded lobes at the base (Safo Kantaka 2004; Lebot 2009).

The inflorescence is a spadix tipped by a sterile appendage, surrounded by a spathe and supported by a peduncle much shorter than leaf petioles (Figure 1.4). The individual flowers are unisexual, small, and without a perianth. Male and female flowers appear on the same spadix (inflorescence) separated by a band of sterile flowers. The male flowers are on the upper part of the spadix - the stamens entirely fused, while the female flowers are at the base of the spadix with a superior one-celled ovary that has an almost sessile stigma. The fruit is a densely packed, many-seeded berry with up to 50 seeds. The seeds are ovoid to ellipsoid, less than 2 mm long, with copious endosperm (Safo Kantaka 2004; Lebot 2009).

Wild and “domesticated” forms of taro occur. The main characteristics of wild *C. esculenta* are long stolons; small, elongated corms; continuous growth; and a predominantly high concentration of calcium oxalate (Figure 1.5) that is associated with acridity (Lebot et al. 2004). Bradbury and Nixon (1998) noted that acridity can be ascribed to an irritant on the raphides that cause a reaction after the raphides puncture the soft skin and mucous membranes. The domesticated taro as well as intermediate types can be either dasheen or eddoe type. These accessions could be hybrids between the two types, or accessions that are difficult to classify because of the unusual shape of their corms (Lebot et al. 2004).

Figure 1.3: A botanical drawing of a taro plant. The large peltate leaves and inflorescences of the taro plant and the stolons and sucker can be seen (Curtis's Botanical Magazine v.120 [ser.3:v.50] 1894; https://ast.wikipedia.org/wiki/Colocasia_esculenta#/media/File: Colocasia_esculenta_CBM.png accessed 20 July 2017)

Figure 1.4: The inflorescence of taro landrace Cocoindia. The yellow spadix is clearly visible. The male flowers and sterile appendage can be seen but the base of the spadix encloses the female flowers. The abaxial side of a peltate leaf with three main nerves can be seen behind the inflorescences (Photo: WS Jansen van Rensburg).

In Asia and the Pacific region, dasheen cultivars are generally diploid and widely distributed in the humid tropics, whereas eddoe cultivars are mostly triploids and are found in subtropical to temperate areas (Matthews 2004). Dasheen is overwhelmingly dominant in both the highlands and lowlands of Papua New Guinea. In Indonesia, dasheen is generally dominant but eddoe occupies highland areas above 1000m (Lebot et al. 2002). Taro is cultivated up to 2500 meters above sea level in tropical latitudes. In

China, dasheen is found in the southern regions because it needs higher temperatures (Xu et al. 2001). In Ethiopia, dasheen is dominant in highland areas and eddoe in lowland areas (Fujimoto 2009). Burkill (1985) and Fujimoto (2009) noted that the eddoe form is in general 'hardier' than the dasheen form and can be grown under 'drier and harsher' conditions. In Asia and the Pacific region, the dasheen type is dominant and the eddoe type is found in some temperate and tropical highland areas. In Africa most taro cultivars are the eddoe type, this may be due to the generally drier African climate in comparison to the climate of Asia and the Pacific region (Safo Kantanka 2004; Fujimoto 2009). Safo Kantanka (2004) noted that eddoe types may have originated in China, where they then spread to the Caribbean region, and then to Africa, indicating a recent introduction of eddoe cultivars. Safo Kantanka (2004) did not speculate on how and when the taro spread to Caribbean and Africa. However, according to Fujimoto (2009), the recent introduction of eddoe cultivars cannot be the case in Ethiopia. Most taro cultivars in Africa, both eddoe and dasheen types, presumably originate from ancient arrivals of tropical Asia, with some later additions. Through time, a diversification of local cultivars and domination of the eddoe type may have taken place, along with development of different cultivation techniques (Fujimoto 2009). In South Africa both eddoe and dasheen type landraces are cultivated in KwaZulu-Natal, however the eddoe type seems to be the most preferred type (Mare 2009). Chaïr et al. (2016) noted that al the South African landraces included in the study were triploid.

Taro is sometimes confused with tannia (*Xanthosoma sagittifolium* (L.Schott)) because of its similar appearance. A ready distinction can be found in the junction of the leaf stalk with the blade, in taro the leaf is peltate with the petiole attached near the centre of the lower surface of the leaf rather than the margin, whereas in *Xanthosoma* the petiole is attached on the leaf margin of the arrow shaped leaves (Safo Kantaka 2004).

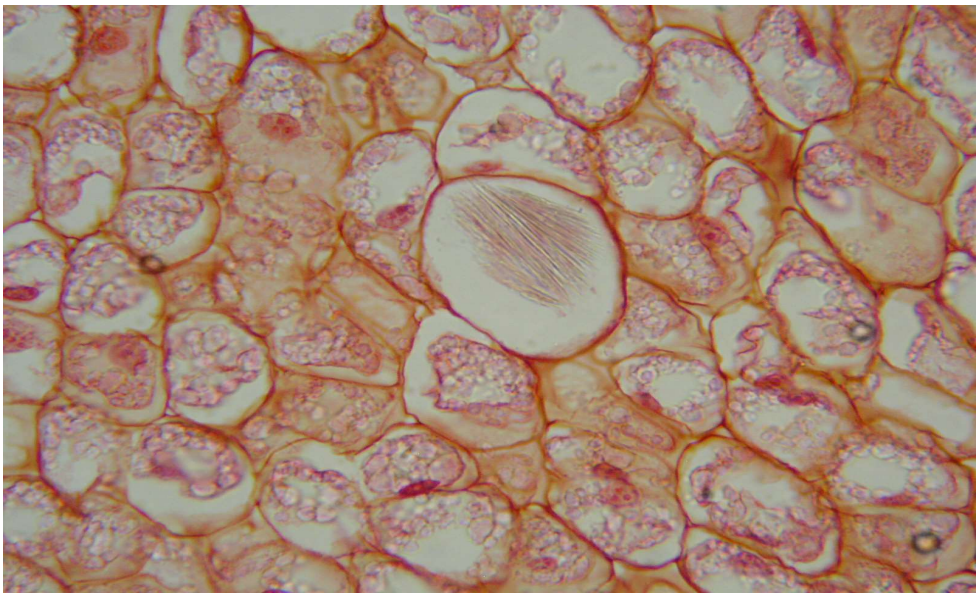

Figure 1.5: Microtome section of a taro leaf stained with safranin. Bundles of oxalic acid crystals can be seen in the big cell in the middle (Photo: L Magadenzane, ARC, unpublished data).

1.1.3 Growth and development

Taro is generally planted in the beginning of the rainy season in most countries; growth of new roots and leaves starts two weeks after planting and the growth of suckers (bulking) begins after two months. Growth of the central corms starts after about two months and in flooded taro after 3–5 months. There is a continuous turnover of leaves and the maximum leaf area and mass is reached after 4–5 months, thereafter leaf stalks become shorter and leaf blades smaller and fewer. Most clones rarely flower and many do not flower at all. Flowering can, however, be induced by treatment with gibberellic acid. Leaf harvesting can start when the plants have about six leaves (approximately three months after planting). Intensive leaf harvesting may reduce corm size, yield and number of suckers. Corms are ready for harvesting 8–10 months after planting under dryland conditions and 9–12 months under wetland conditions, although the corms will reach their maximum mass a few months later (Safo Kantaka 2004; Lebot 2009).

Taro is propagated vegetatively. It is sometimes difficult to keep planting material in a healthy condition during the dry season or periods of drought. Essentially four types of planting material are used; side suckers growing from the main corm, small unmarketable cormels (60–150 g), corm pieces, and head setts or 'huli', i.e. the apical 1–2 cm of the main corm with 15–20 cm of the leaf stalks attached. In Ghana, planting is mainly by use of either young suckers or mature setts cut from harvested corms. Planting material must be taken from healthy plants (Safo Kantaka 2004; Lebot 2009).

1.1.4 Origin and geographic distribution

Taro is probably one of the oldest crops and has been grown for more than 10 000 years in tropical Asia (Lebot 2009). It is believed that taro was domesticated in northern India, but independent domestication in New Guinea has also been reported. *Colocasia esculenta* occurs wild in tropical Asia, extending as far east as New Guinea and northern Australia. A form with long stolons, which occurs throughout this region, has been postulated as the ancestor of cultivated taro on the basis of ribosome-DNA analysis (Safo Kantaka 2004). Eddoe types may have originated in China, from where they spread to the Caribbean region, and then to Africa (Safo Kantaka 2004; Lebot 2009). It was spread by human settlers eastward to New Guinea and the Pacific over 2000 years ago, where it became one of the most important food plants economically and culturally (Safo Kantaka 2004; Lebot 2009). Distribution to China, Egypt and East Africa also occurred at least 2000 years ago. Taro was taken to West Africa from Egypt and East Africa by the Arabs. It was introduced into Europe from Egypt. From Spain it was taken to the New World and new introductions may have been made into West Africa from tropical America. Presently, taro is grown in many tropical and subtropical areas around the world for its corms, leaves and flowers (Safo Kantaka 2004; Lebot 2009:281).

There is an indication that when taro was introduced to a new area, only a small fraction of genetic variability in heterogeneous taro populations was transferred, possibly

causing random differentiation among locally adapted taro populations (Sharma et al., 2008). Nguyen et al. (1998) showed that the Yunnan area might be an important area in the evolution and dispersal of taro. However, Ivancic and Lebot (1999) are of the opinion that the centre of origin will never be found for certain because considerable genetic diversity has been lost already.

1.1.5 Utilization and nutritional value

Taro is a staple food crop in the Pacific Island countries and parts of Asia (Opara, 2003; Lebot and Aradhya 1991). The leaves, petioles, flowers, corms and cormels are used, the corms and cormels being most popular.

Certain taro varieties are valued for multiple uses such as food, feed, medicine and ritual purposes (Hue et al. 2003). The corms of taro are eaten boiled, fried or roasted as a side dish or are used for making 'fufu', a starch staple made from boiled and pounded root vegetables. Dasheen type taro is comparatively mealy, whereas in eddoe types the cormels have a more firm structure and taste somewhat nutty. The corm is also sliced and fried into taro chips and used in the preparation of soups, beverages and puddings. In Hawaii the corms are processed into flour which is used for biscuits and bread. Throughout the Pacific Islands, they are also boiled and made into a paste that is left to ferment to produce 'poi'. The Chinese feed corms and leaves from wild types and inferior varieties to their pigs (Safo Kantaka 2004; Fujimoto 2009).

Taro leaves and leaf stalks are used as a leafy vegetable and potherb for soups and sauces, or as relish. They are especially popular in parts of West Africa, north-eastern India and the Caribbean region. The leaves and leaf stalks contain oxalic acid, which causes itchiness in the mouth and throat, but cooking denatures acridity. Leaves and leaf stalks of the dasheen type seem to be less acrid than those of the eddoe type. The stolons that are formed in some types are eaten too (Safo Kantaka 2004; Matthews

2004). It's reported that the flowers are consumed in China (Jianchu et al. 2001; Matthews 2004) and Bangladesh (Paul et al. 2011). Taro leaves are also used as temporary wrapping for small articles such as spices, herbal medicines, and wild honey (Fujimoto 2009).

Taro corms, stolons and leaves are used as fodder for pigs (Safo Kantaka 2004; Hue et al. 2003; Fujimoto 2009). Besides its nutritional value, taro is traditionally used as a medicinal plant and provides bioactive compounds which act as immune stimulators (Pereira et al. 2015). Taro is also used medicinally for headaches (Hue et al., 2003) gastro-intestinal disorders and dental decay in children (Safo Kantaka 2004).

Taro corms are an excellent source of carbohydrates and potassium (Manner and Taylor, 2010; Oke 1990). The nutritional content for taro corms varies between genotypes (Guchait et al. 2008). Mare and Modi (20212) noted that planting date and fertilizer. Furthermore, they also noted that interaction between temperature, packaging, landrace (genotype) and sampling date influence reducing sugars during storage. Mineral content plays a crucial role in consumers' acceptance according to Champagne et al. (2013). The digestibility of taro starch is very high and the starch grain is about ten times smaller than a starch grain of potato, it is therefore suitable for people with digestive problems. Taro is an excellent food for diabetics because the low glycemic index facilitates slow release of glucose into the bloodstream (Manner and Taylor 2010). Taro starch is hypoallergenic, making it useful for people allergic to cereals, it is even used as substitute baby food for infants with milk sensitivity (Safo Kantaka 2004; Darkwa and Darkwa 2013). Interaction between landrace (genotype), planting date and fertilizer application influence starch content in corms (Mare and Modi 2012). They also noted that the interaction of temperature, packaging, cultivar and sampling month influence starch content. Taro flour has been reported to have been used in infant food formulae and canned baby foods in the United States of America (Darkwa and Darkwa 2013).

Yellow fleshed taro contains higher levels of β-carotene than white flesh (Engelberger et al. 2003). Taro is an ideal crop to help in combatting hunger and malnutrition die to the highly digestible, low GI starch particles and the availability of germplasm with higher β-carotene and flavonoids. However, the yields are relatively low and taro is more adapted to tropical and sub-tropical climates. It creates an opportunity to breed for higher yields and plants that are adapted to more arid conditions.

All parts of most cultivars are acrid, though the acridity in taro is not due to the calcium oxalate raphides. Some irritant on the raphide surface caused the acridity, with the raphides apparently functioning to carry the acridity factor (Paul et al. 1999). Cooking the taro generally denatures the acridity (Manner and Taylor 2010).

1.1.6 Production and international trade

World production of taro increased from 4 487 124 tonnes in 1961 to 10 108 223 tonnes in 2014 according to the FAO (2017). The area under production increased from 758 228 hectares to 1 455 508 hectares during the same period (FAO 2017). In 2014 Africa produced 7 314 417 tonnes of taro. The biggest production was in Western Africa (4 798 185 tonnes), followed by Central Africa (1 966 283 tonnes), Eastern Africa (427 116 tonnes) and Northern Africa (122 833 tonnes). Other areas that produced significant amounts of taro in 2014 are Asia (225 9532 tonnes), Central America (83 331 tonnes), Oceania (42 5247 tonnes) and Melanesia (38 5370 tonnes). Nigeria specifically had the highest production of taro in 2014 with 3 273 000 tonnes produced from 639 980 hectares. Nigeria is followed by China with a production of 1 884 987 from 97 601 hectares. Cameroon and Ghana followed with a production of 1 672 731 tonnes and 1 299 000 tonnes respectively (FAO 2017). No production figures were available for South Africa.

It is difficult to get exact producer price data, but the average producer price for a tonne of taro was 975.17USD in 2014. The producer price varies from 186.6USD/tonne in Egypt to 2 794.9 USD/tonne in Japan (FAO 2017).

In some regions of Asia and the Pacific, taro is being been gradually replaced by more productive root crops such as tannia (*Xanthosoma sagittifolium*), cassava (*Manihot esculenta* Crantz) and sweet potato (*Ipomoea batatas* (L.) Lam.). This is leading to the genetic erosion of variability in taro (Safo Kantaka 2004; Caillon at al. 2006; Fujimoto 2009).

Taro corms and leaves, although common in local markets, are mostly grown for subsistence and home consumption. Large-scale commercial production is not common. Local consumption forms the greatest utilisation of taro produced on other continents too. However, small amounts are exported to Europe and Australia for the immigrant community. Trinidad and Tobago also import some taro (Safo Kantaka 2004).

1.1.7 Diseases and pests

Taro blight (*Phytophthora colocasiae*) is a major wetland taro disease, causing purple to brown circular water-soaked lesions. It is the most devastating taro disease, particularly in the Pacific region where it has caused considerable losses due to rot. Taro blight is associated with high relative humidity. Several species of *Pythium* (*P. adhaerens, P. aphanidermatum, P. arrhenomanes, P. carolinianum, P. debaryanum, P. delicense, P. graminicola, P. helicoides, P. irregular, P. middletonii, P. myriotylum, P. splendens, P vexans* and *P ultimum*) cause taro soft rot, with wilting and chlorosis of leaves. Sclerotium rot caused by *Sclerotium rolfsii* is characterized by stunting of the plant, rotting and formation of many spherical sclerotia on the corm. In both flooded and upland taro, dark brown spots that appear in older leaves are caused by *Cladosporium*

colocasicola and *Phyllosticta colocasiae* (Jackson 1985; Safo Kantaka 2004; Revil et al. 2005; Lebot 2009).

Dasheen mosaic virus (DsMV) and other viruses have been reported, but are seldom serious. In the Pacific region, the alomae virus disease causes serious damage. Symptoms start with a feathery mosaic on the leaves followed by crinkling and formation of outgrowths on the surface after which, the entire plant becomes stunted and dies. Alomae disease is caused by the combined infestation from the taro large bacilliform virus (TLBV) and the taro small bacilliform virus (TSBV). Presence of only TLBV results in a milder form of the disease called 'bobone'. The viruses are transferred by grasshoppers (*Gesonula zonocera mundata* Navas) and mealy bugs (*Pseudococcus longispinus*), respectively, but not by mechanical contact. Taro vein chlorosis virus, and Taro reovirus also occur in the pacific (Safo Kantaka 2004; Revil et al. 2005; Lebot 2009).

Attack by root-knot nematodes (*Meloidogyne* spp.) can result in considerable crop loss and insect pests on taro may cause serious damage. Damage by *Hercothrips indicus* thrips is shown as a silvery discoloration of the leaves and can result in severe leaf shedding. Adult taro beetles (*Papuana* spp. e.g. *Papuana huebneri* and *Papuana woodlarkiana*) tunnel in the corm up to the growing point. Young plants wilt and die but older plants usually recover. This pest is reported in the Pacific and South-East Asia, but not in Africa. Larvae of the sweet potato hawk moth (*Agrius convolvuli*) defoliating the plant reduces corm quality (Safo Kantaka 2004; Lebot 2009).

1.1.8 Yield

The yield of leaves is not recorded and the corm yields are variable depending on production area, agronomic practices and genotype. The average yield on a world basis is 5–6 t/ha, but a good crop on fertile soil gives at least 12 t/ha, and yields of higher than

40 t/ha have been achieved in Hawaii (Safo Kantaka 2004). The average global yield increases from 5.9 t/ha in 1961 to 6.9 t/ha in 2014 (FAO 2017).

At a regional level, the average yield during 2014 was 16.6 t/ha in Asia, 10.25 t/ha in Central America, 9.7 t/ha in America, 9.6 t/a in the Caribbean, 8.2 t/ha in Melanesia, 7.8 t/ha in Oceania, 6.1 t/ha in South America, 5.8 t/ha in Africa and 5.1 t/ha in Polynesia. Within Africa, the highest yields were reached in Northern Africa (34.82 t/ha), followed by Central Africa (7.9588 t/ha) and Eastern and Western Africa (5.2 t/ha) (FAO 2017).

The highest yields, during 2014, were recorded in Egypt (34.8 t/a), Cyprus (26.1 t/a) and mainland China (19.3 t/a). Although Nigeria is the biggest producer, the average yield in Nigeria was only 5.1 t/a during 2014. In Ethiopia the yield can vary between 1.79 kg/m^2 (1.26 kg/plant) and 1.00 kg/m^2 (0.65 kg/plant) for the Highlands and lowlands respectively (Fujimoto 2009). However, Lebot (2009) reported that yields of 60-110t/ha have been recorded under traditional cropping systems. At the ARC Research Station, Roodeplaat (South Africa) yields vary between 6 and 10 t/ha (Personal communication Abe Shegro Gerrano). Mare (2012) noted that landrace, agronomic practices influence the yield.

1.1.9 *Colocasia esculenta* in South Africa

Taro is being cultivated in South Arica for a long time, but no information exists on how and when taro was introduced. Taro is cultivated in the subtropical eastern side of South Africa. It is cultivated as far south as Bizana in coastal Eastern Cape Province, then northwards on the coastal areas of KwaZulu-Natal and certain areas of Mpumalanga and Limpopo Provinces (Modi 2004; Shange 2004). Subsistence and small scale farmers in South Africa mostly cultivate taro for own use and trade on the informal market (Figure 1.1 and 1.2) (Shange 2004). No improved cultivars exist but Mare (2006) farmers

were able to distinguish up to five landraces. Some farmers do produce taro for the formal market at a very small scale (Modi 2003). The planting season for taro in South Africa is from August to October, and harvesting takes place six to eight months later during April to May (Shange 2004; Mare 2006). Taro is mostly cultivated under dryland conditions; however, a small portion of wetland production occurs in the northern parts of KwaZulu-Natal (Shange 2004). Organic production is practiced by most of the farmers (Modi 2003), who also practice mixed cropping with sweet potatoes, beans, maize, potatoes and peanuts.

1.2 Genetic Diversity

The existing variation, due to genetic differences, within a population or species is called genetic diversity. Genetic diversity is important for the survival and adaptability of a species. Species with high genetic diversity will produce a wider range of offspring. Some of the offspring will be better adapted than others. Genetic diversity, therefore, facilitates populations or species adaptation to changing environments (Devi 2012; NBII 2017). Genetic diversity within and between populations or species can be assessed using various parameters and methods such as:

- agro-morphological performance under uniform environmental conditions (growth habit, stolon formation, plant height, shape, colour and orientation of lamina, maturity, shape and weight of corms and cormels, corm and cormel yield, flesh colour and edibility of tubers, resistance against leaf blight etc.),
- biochemical traits (protein expression profiles and isozymes) and
- Cytological and DNA markers (e.g. RAPD, AFLP, SSRs etc.) (Devi 2012).

Over the past years, several studies reported on the genetic diversity of taro. The earliest studies use agro-morphological descriptors and many researcher still do rely on agro-morphological descriptors, especially to characterize and evaluate germplasm and breeding lines in breeding projects. Isozymes were very popular in the late 1990s, but is

still being used because it is relatively easy and affordable. DNA based methods have gained popularity lately because of the reproducibility and the relative large amounts of data that can be generated.

1.2.1 Agro-morphological characterization

Agro-morphological characterization is a key component of traditional breeding programs. Agro-morphological characterization is the use of agricultural characteristics such as yield, and morphological characteristics such as flower colour to describe and measure genetic diversity and variability within a population or species (Ivancic and Lebot 1999). Hartati et al. (2001), Jianchu et al. (2001), Hue et al. (2003), Okpul et al. (2004), Quero-Garcia et al. (2004) Caillon et al. (2006), Sing et al. (2008), Trimanto et al. (2010), Sing et al. (2011), Orji and Ogbonna (2015) and Mwenye et al. (2016) have used agro-morphological characteristics to study genetic diversity in taro.

Taro exhibits a wide array of agro-morphological variation. Numerous variable, but stable, morphological traits exist and are used as descriptors for varietal identification and assessment of genetic diversity. Bioversity International developed a descriptor list for taro (IPGRI 1999). The list includes 73 descriptors, including four general plant habit descriptors, 20 leaf and petiole descriptors, 15 inflorescence descriptors, six seed and fruit descriptors, 12 corm, four cormel and two root and corm descriptors (IPGRI 1999). Many of these descriptors are highly technical and if an accession does not flower naturally, the flower, fruit and seed descriptors can only be assessed if flowering is induced. Subsets of the IPGRI list of descriptors were used by Okpul et al. (2004) and Singh et al. (2008) to describe the morphological variation and perform diversity analysis. These authors used 18 and 30 descriptors respectively. Okpul et al. (2004) cautioned against the use of colours or pigmentations and their patterns on leaf petioles and corm flesh because the inheritance of pigments in taro is not clear, as it seems to be influenced by different methods of vegetative propagation. Furthermore, corm shape depends

strongly on location, environmental conditions, and plant age (Ivancic and Lebot, 1999). Mare (2006) use ten qualitative morphological characteristics to characterise South African taro landraces in KwaZulu-Natal with the help of farmers.

The number of descriptors used vary between the different studies. The IPGRI descriptor list (IPGRI 1999) is time-consuming, but generate large amounts of data. The condensed descriptor list used by Singh et al. (2008) has less characteristics, but were still able to identify duplicates. The much shorter list used by Mare (2006) is easy to use and include important consumer characteristics like taste, cooking time and sliminess.

Taro cultivars are vegetatively propagated, therefore, low intraspecific variability is expected (Okpul et al. 2004). Nevertheless, Okpul et al. (2004) observed high morphological variation in Papua New Guinea germplasm by using 18 agro-morphological descriptors. This is in agreement with results of studies by Lebot et al. (2000) and Godwin et al. (2001). According to Okpul et al (2004) this variability may be attributed to sexual recombination, migration and mutation, with subsequent selection by farmers in geographical isolation for adaptability under various agro-ecological regimes and cropping systems and culinary and quality preferences.

Quero-Garcia et al. (2004) and Okpul et al. (2004) did not find any significant correlations and patterns in Vanuatu taro germplasm diversity using morphological characteristics. This may be because the characters used were too heterogeneous (passport, agronomic and morphological characters), and generally not correlated. However, no clearly differentiated groups were produced when working with agronomic and morphological characters separately. Accessions with rare traits (i.e., orange corm colour) appeared clearly isolated in the dendrograms (Quero-Garcia et al. 2004).

Quero-Garcia et al. (2004) identified duplicates in the Vanuatu germplasm collection using agro-morphological markers. Singh et al. (2008) used a subset of thirty agro-morphological characteristics to rationalise the Papua New Guinea taro germplasm collection (Singh *et al.* 2008). Variation in some of the agro-morphological traits is depicted in Figure 2.2.

1.2.2 Isozymes

Isozymes or isoenzymes are multiple forms of enzymes that differ in amino acid sequence but catalyse the same chemical reaction. These enzymes usually display different kinetic parameters or different regulatory properties. Lebot and Aradhya (1991), Isshiki et al. (1998), Nguyen et al. (1998), Ivancic and Lebot (1999), Lebot et al. (2000), Hartati et al. (2001) and Trimanto et al. (2010) used isozymes to study diversity in taro.

Nguyen et al. (1998) used esterase and revealed large diversity in the esterase isozyme in 69 taro accessions from Nepal, Thailand, Yunnan, Ryukyu and other places in South Eastern Asia. Isshiki et al. (1998) used glucose-6-phosphatase isomerase, shikimate dehydrogenase, isocitrate dehydrogenase, and two forms of aspartate aminotransferase. They were able to differentiate between 58 Japanese diploid and triploid taro cultivars. The Japanese cultivars also have a very narrow genetic base. Isshiki et al. (1998) also established that the triploid cultivars did not originate as bud mutations or hybridization between Japanese diploid cultivates.

Isozyme studies by Lebot and Aradhya (1991) used seven polymorphic enzyme systems (MDH, IDH, PGI, 6-PGD, ME, SkDH, and ADH) and revealed the existence of two germplasm pools, one in southeast Asia and the second in Melanesia, indicating the possibility of two independent domestication processes.

Ivancic and Lebot (1999) were able to distinguish between wild type taro and taro cultivars in New Caledonia using peroxidase, esterase, shikimic-dehydrogenase and phosphoglucomutase. The wild types of taro were not closely related to New Caledonian and Pacific cultivars. Ivancic and Lebot (1999) suggested that, in light of the physical isolation of New Caledonia, the Caledonian cultivars were probably introduced as clones from other islands, such as Vanuatu, by early Melanesian migrants. The wild types appear to be genetically distant from other Melanesian wild taros.

Hartati et al. (2001) used phosphoglucoisomerase, malate dehydrogenase, isocitric dehydrogenase, 6-phosphogluconic dehydrogenase, shikimic dehydrogenase and malic enzyme to determine the genetic diversity in Indonesian germplasm. They reported no correlation between isozyme and morphological characterization; these results supported the earlier findings by Lebot and Aradhya (1991).

Lebot et al. (2004) used malate dehydrogenase, phosphogluco-isomerase, isocitrate dehydrogenase, 6-phosphogluconate dehydrogenase, mallic enzyme and shikimic dehydrogenase and proved that Indonesia, Malaysia, Thailand and Vietnam host significant allelic diversity. In comparison, the countries located in the Pacific (the Philippines, Papua New Guinea, Vanuatu) appear to assemble limited allelic diversity. The results of Lebot et al. (2004) indicated a narrow genetic base, especially in the Pacific islands.

1.2.3 DNA markers

Various DNA markers were used to determine genetic diversity in taro (Lebot, 2009:313). These include random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP).

1.2.3.1 RAPDs

Random Amplified Polymorphic DNA (RAPD) markers are DNA fragments from PCR amplification of random segments of genomic DNA, with single primer of arbitrary nucleotide sequence. Irwin et al. (1998), Hartati et al. (2001), Lakhanpaul et al. (2003), Sharma et al, (2008), Singh et al. (2011) and Das et al. (2015) made use of RAPDs to study the genetic diversity of taro.

Forty-four accessions of diverse origins (Melanesia, Indonesia and Polynesia) were analysed with RAPD markers but show no clear geographical or morphological correlation; however, the analysis revealed that the Melanesian and Indonesian taros are far more diverse than the cultivars from Polynesia (Irwin et al. 1998). Lakhanpaul et al. (2003) also did not find any strict relationship between the clustering pattern and geographical distribution, morphotype classification and genotypic diversity. Lakhanpaul et al. (2003) also observed that accessions classified as belonging to the same morphotypic group did not always cluster together. In contrast, Sharma et al. (2008) observed that accessions form northern and southern India tend to cluster together in two distinct clusters.

1.2.3.2 SSRs

Simple sequence repeats (SSR), or microsatellite polymorphisms, are tracts of repetitive DNA in which certain DNA motifs (ranging from 2–6 base pairs) are repeated, typically 5–50 times. Microsatellites occur at thousands of locations within an organism's genome and have a higher mutation rate than other DNA areas leading to high genetic diversity. Mace and Godwin (2002), Noyer et al. (2004), Singh et al. (2008), Hu et al. (2009), Sardos et al. (2011), Singh et al. (2011), Lu et al. (2011), You et al. (2014) and Chaïr et al. (2016) have used SSRs to study genetic diversity in taro.

Microsatellite and SSR markers were tested on 17 accessions from several Pacific countries (Mace and Godwin, 2002). They proved to be a valuable tool for the identification of duplicates, although the geographical structure produced was not very informative, probably due to the size of the sample and the low number of primers used. You et al. (2014) also proved that SSR markers were able to distinguish between 68 taro cultivars. Similarly, Quero-Garcia et al. (2006) did not reveal any clear geographical structure and Caillon et al. (2006) observed that genetic diversity cultivated in one village was equivalent to the overall genetic diversity cultivated within Vanuatu. In Vanuatu, Sardos et al. (2011) distinguished between genotypes by SSRs and observed that genetic clusters are mainly differentiated by rare alleles. In contrast to other researchers, Sardos et al. (2011) did find a degree of correlation between geographical and present social and genetic diversity. SSRs were able to discriminate between diploid and tetraploid germplasm (Chaïr et al. 2016).

1.2.3.3 AFLPs

Amplified fragment length polymorphism (AFLP) use restriction enzymes to digest genomic DNA with adaptors are then ligated to the sticky ends of the restriction fragments. A subset of the restriction fragments is then amplified using primers complementary to the adaptor sequence, the restriction site sequence and a few nucleotides inside the restriction site fragments. Kreike et al. (2004), Quero-Garcia et al. (2004), Lebot et al. (2004), Caillon et al. (2006), Sharma et al. (2008) and Mwenye et al. (2016) used AFLPS to study diversity in *C esculenta*. Sharma et al. (2008) found that Indian taro cultivars can be distinguished from each other using AFLPS. Quero-Garcia et al. (2004) identified no duplicates with AFLP markers.

Kreike et al. (2004) used AFLP markers to study the diversity of a core sample of accessions from seven different countries. Most accessions could be clearly differentiated by using three primer pairs and few duplicates were identified.

Differentiation between Southeast Asian and Melanesian taros was obtained confirming the isozyme results (Kreike et al. 2004). Kreike et al. (2004) also revealed that the diversity among wild types was greater than that within the cultivated taro. Quero-Garcia et al. (2004) used AFLP analysis in Vanuatu to validate a stratification methodology of large germplasm collections. Quero-Garcia et al. (2004) demonstrate that AFLPs were able to differentiate between all the accessions and no duplicates were identified, even in geographically different but almost morphologically identical accessions. Quero-Garcia et al. (2004) also reported that the AFLP variability did not show any geographic pattern. Mwenye et al. (2016) noted low levels of diversity within Malawi, with correlation between geographical location and diversity.

1.2.4 Karyotype analysis and cytogenetics

Nguyen et al. (1998) have identified both diploid and triploid accessions from Nepal, Thailand, Yunnan, Ryukyu and other locations in South Eastern Asia. According to Yen and Wheeler (1968), Kurvilla and Singh (1981), Coates et al. (1988), and Matthews (1990), the majority of Pacific genotypes should be diploids, with most of the triploids existing in Asia (Ivancic and Lebot 1999).

1.2.5 Correlation between the different methods

Sharma et al. (2008) demonstrated that RAPDs revealed higher levels of genetic variation than isozymes and that isozyme dendrogram has poorer discriminating power between accessions than RAPD dendrograms. Sharma et al. (2008) noted that one possible explanation; isozyme variation only reflects differences in protein-coding genes and coding sequences are under a greater selection pressure to maintain functional sequences. RAPDs on the other hand can detect variation in both coding and non-coding regions. Similarly, Singh et al. (2011) observed a correlation between results obtained with morphological traits, RAPDs and SSRs. Trimanto et al. (2010) detected high correlation between isozyme data and morphological data. However, Hartati, Prana and

Prana (2001) found no clear correlations on dendrograms based on morphological characteristics, isozymes and RAPDS. Lebot and Aradya (1991) also reported no correlation between the dendrograms produced by morphological and isozyme data while Nguyen et al. (1998) reported no correlation between esterase isozymes and geographic distribution (except for the Nepalese accessions) and ploidy level.

Jianchu et al. (2001) found correlations between folk taxonomy and uses, and morphotypes based on ethnobotanical, agro-morphological, and preliminary genetic characterization. Noyer et al. (2004) observed correlation between the dendrograms from their SSR markers and that of Kreike et al. (2004) based on AFLP markers. Noyer et al. (2004) observed differentiation between Southeast Asian and Melanesian taros confirming AFLP and isozyme results. Accessions from Thailand are grouped, but Indonesian accessions did not grouped together, further confirming AFLP results (Noyer et al. 2004).

1.2.6 Genetic diversity in taro

The genetic diversity for taro seems to be large in South East Asia but small in Africa and the Pacific region. (Safo Kantaka 2004; Lebot 2009; Paul et al. 2011; Orji and Ogbona 2015; Chaïr et al. 2016). South-East Asia (Indonesia, Malaysia, Thailand and Vietnam, Bangladesh, Japan and New Guinea) hosts significant allelic diversity (Isshiki et al. 1998; Lebot et al. 2000; Safo Kantaka 2004; Lebot 2009 and Paul et al. 2011); whereas Pacific Countries (the Philippines, Papua New Guinea Vanuatu) (Lebot et al. 2000; Safo Kantaka 2004; Lebot 2009 and Paul et al. 2011) and Africa (Safo Kantaka 2004; Fujimoto 2009; Lebot 2009; and Mwenye et al. 2016) appear to have limited allelic diversity. In Africa, the genetic diversity is slightly higher in Madagascar and Madeira than in South Africa, Ghana and Burkina Faso (Chaïr et al. 2016).

Clonally propagated crops, like taro, tend to have a narrow genetic base. The wide genetic diversity of taro in certain places can be attributed to the fact that certain taro cultivars do flower and are cross pollinated by naturally occurring pollinators. Cross compatibility between species occurs (even with wild types) and insect pollinators do occur abundantly in certain areas (Hartari et al. 2001). Mare (2006) noted that there might just be four taro landraces in central KwaZulu-Natal, South Africa in spite of the taro's long history in South Africa. This might be due to the fact that taro is vegetatively propagated in South Africa. Flowering seldom occur and the known natural pollinators do not occur in South Africa.

Ivancic and Lebot (1999), Hartati et al. (2001), Jianchu et al. (2001), Matsuda and Nawata (2002), Hue et al. (2003), Kreike et al. (2004) Caillon et al. (2006) and other authors observed no correlation between geographic distribution and diversity of taro. However, Sharma et al. (2008) noted correlation between cluster analyses (but not dendrograms) based on RAPDS and geographic distribution. They also traced evidence of local natural selection. Sharma et al. (2008) reported high levels of diversity in Indian taro collection and attributed the largest portion of the diversity to geographic isolation.

The low genetic diversity in Africa and the Pacific areas have certain implications on breeding of taro in these areas. One of these is the introduction of germplasm from other areas. These areas is also outside the centre of diversity of taro and incidences of natural hybridization is low.

Very little is known about the genetic diversity of taro in South Africa. Mare (2006) noted that local the farmers are able to distinguish between different landraces. Mabhaudhi and Modi (2013) distinguished between three taro landraces using agro-morphological characteristics and SSRs. More information is needed to understand the genetic structure, of taro in South Africa, better

1.3 Breeding in Taro

There are three approaches to obtain improved cultivars of taro (Sivan and Liyanage 1993). The easiest is to collect and evaluate local germplasm in order to identify promising lines to propagated and distributed. Alternatively, elite cultivars can be imported from other countries to evaluate under local conditions, to identify cultivars suitable for local conditions and markets. Lastly, controlled breeding can be used to recombine characteristics in progeny that are evaluated against a set of predetermined criteria (Sivan and Liyanage 1993).

The discovery of methods of flower induction in taro has greatly facilitated breeding (Safo Kantaka 2004). One of the first breeding programmes was initiated in the early 1970's in the Solomon Islands to breed for taro leaf blight resistance (Patel *et al*. 1984, as cited by Lebot 2009). This was followed by breeding programmes in Hawaii, Samoa, Papua New Guinea (PNG), India, Philippines, Fiji and Vanuatu (Lebot 2009). There are taro breeding programmes in Mauritius that used mutation breeding to identify taro blight resistance (Seetohul et al. 2007). Lebot (2009) also noted that little was achieved in these programmes due to the narrow genetic base of the breeding stock and the introduction of "wild" germplasm that also introduced undesirable traits.

Most domesticated taro genotypes do not flower naturally (Del Peno 1990; Wilson 1990; Lebot 2009). Wild types do flower more easily and the character can be bred into a population (Lebot 2009). Lebot (2009) lists several possible ways to promote flowering in taro and other aroids. These are treatment with gibberellic acid (0.3 – 0.5 g/ℓ), removal of leaves (effective for *Xanthosoma* and *Alocasia*), heat and drought stress, and removal of cormels and stolons. However, spraying the parental material with GA was considered the most efficient and reliable method (Ivancic 1992 as cited by Iramu et al. 2009; Wilson 1990; Mukherjee et al. 2016). The first inflorescences appear from 60 to 90 days after

gibberellic acid application, depending on the clone and the growing conditions (Wilson 1990).

Plants produce a floral bract or flag leaf before the plant produces an inflorescence. These bracts are produced by both natural and induced flowering (De la Pena 1990; Wilson 1990; Lebot 2009). The first inflorescences usually appear within 1–3 weeks after the flag leaf. Gibberellic acid induces deformities before the normal inflorescences. These deformities include incomplete and patches of floral colour and texture on the leaves. Gibberellic acid also stimulates plants to produce more suckers, more stolons, elongated petioles, and branching corms (Wilson 1990).

Taro flowers are thermogenic. The flowers have a distinct odour when female flowers are receptive (Wilson 1990). Taro flowers are protogynous, thus the female flowers become receptive before the pollen is shed from the male flowers from the same inflorescence (Mukherjee et al. 2016) however, Wilson (1990) noted that the female flowers may be receptive on the same day as the pollen shed of the male flowers in the some inflorescence or it may occur a day before or even after, depending on the location and genotype. The two sides of the spathe enclosing the base of the inflorescence “crack open” and the constricted part of the spathe becomes loose around the band of sterile flowers (Wilson 1990) as the spathe of the inflorescence unfold slowly and enable pollinators to enter. The majority of insects will remain inside the inflorescence until the next morning when the inflorescence will be completely opened and the pollen released. The odour will disappear but the same attraction will come from another inflorescence, which will release pollen a day later (Lebot 2009). The crack closes after pollen shed and the spathe becomes tight around the band of sterile flowers (Figure 1.6) (Wilson 1990).

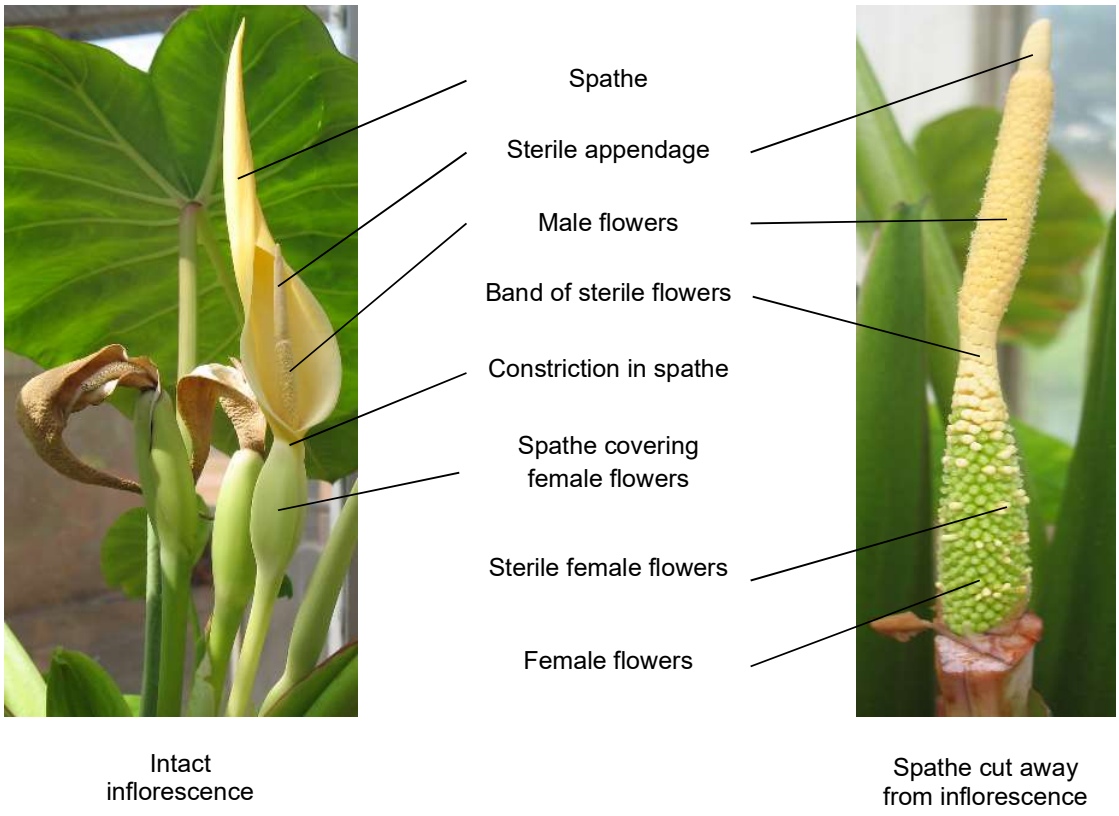

Figure 1.6: The taro inflorescence. The complete inflorescence from Cocoindia on the left. The spathe in the inflorescence from a line 2-2 on the right was cut away to show the female flowers (Photos: WS Jansen van Rensburg).

Pollination is done by various insects, wind or water, though insects are the main vectors (Lebot 2009). Insects that pollinate taro naturally include Drosophilidae (mainly *Drosophila pisticola* and *D. stamenicola*, in Papua New Guinea) (Lebot 2009), dipteran flies, like *Dacus dorsalis,* in Malaysia, (Lebot 2009) and bees, small solitary wasps, small *Coleoptera* insects, mosquitoes and ants (Lebot 2009) no natural pollinator was observed in Africa. Wind pollination is significant for genotypes with open flowering and a fully exposed male part of the spadix (Figure 1.6). Rain results mostly in self-fertilization by washing pollen grains from the male part of the spadix to the female part (Lebot 2009).

Figure 1.7: Taro fruiting body with numerous berries. The colours vary from green to yellow, orange and almost black (Lebot 2011).

For controlled pollination, the stigma becomes receptive at the time when the inflorescence emerges from the petiole sheath, about five days before the odour is released or six days before pollen is shed, and remains in this condition for up to 10 days (Okpul and Ivancic 1995). On sunny days in New Caledonia, pollen appeared before 08:00 in vigorous populations along rivers, but in drier places pollen could be seen after 10:00 (Ivancic and Lebot 1999). Ivancic and Lebot (1999) also found that pollen remain viable for up to 18 days in New Caledonia. Asynchrony in flowering during artificial hybridization can be overcome by cryostoring of pollen (Mukherjee et al. 2016).

The taro fruit is a cluster of densely packed berries (Figure 1.9). Each berry contains 1 to 10 seeds, but it may contain up to as many as 28 to 35 seeds (Wilson 1990; Iramu et al., 2009). The fruits are ready to be harvested 30–35 days after fertilization. Taro seeds

are tiny, less than 2 mm long. When mature they are ovate in shape, hard, and conspicuously ridged longitudinally. The seeds germinate in 7 to 14 days with no apparent dormancy. Seeds can be stored for one year in a moderately cool and dry room. They can remain viable for at least two years in a desiccator inside a refrigerator (Wilson 1990). Dry winds and high temperatures often cause seed set failure (Lebot 2009; Mukherjee et al. 2016).

The aim of most taro breeding programmes is yield (Sivan and Liyanage 1993; Soulard et al. 2016), quality (Sivan and Liyanage 1993; Iramu et al., 2009) and pest and disease resistance (Sivan and Liyanage 1993; Iramu et al., 2009). Many taro breeders emphasis yield in the early generations of taro breeding programmes according to Soulard et al. (2016). Several specific characters were evaluated in a breeding programme. The most important characteristics are plant characters (plant type, petiole colour), plant vigour, sucker number (Sivan and Liyanage 1993), resistance to pests and diseases (viruses, fungi, and insects) (Sivan and Liyanage 1993; Seetohul et al. 2007) maturity, marketable and non-marketable yield, corm characters (shape, smoothness, colour of buds, basal rings, petiole base and flesh) and eating quality (dry weight percentage, specific gravity, taste) (Sivan and Liyanage 1993). According to Sivan and Liyanage (1993), it will take six to ten years to release a taro cultivar using traditional breeding methods. Recently, emphasis has also been placed on the nutrient composition of taro corms. Breeding is done to increase the nutrient content (bio-fortification), or decrease the anti -nutrient content of taro. These compounds are beta-carotene, anthocyanin antioxidants, phenolic compounds and oxalates etc. (Guchhait 2008; Champagne et al. 2013).

The presence of stolons was found to be often associated with undesirable traits such as poor corm shape, poor taste quality and acridity (Lebot et al. 2004). Heritability values compared to narrow-sense heritabilities, suggest a possibility of using family selection in the first cycles of a breeding programme (Lebot 2009). Orji and Ogbonna (2015) and

Soulard et al. (2016) found a strong indication that stolons, suckers and flowering are under genetic control. Stolon production and the number of suckers are strongly negatively correlated, while flowering and the number of inflorescences are not correlated to any other traits (Soulard et al. 2016). Orji and Ogbonna (2015) noted that plant girth was positively correlated to plant height but negatively correlated to the number of suckers. The number of leaves and the number of suckers are also positively correlated (Orji and Ogbonna 2015). Dry matter content was negatively correlated to fresh weight (Quero-Garcia et al. 2006; Mulualem and WeldeMichael 2013; Soulard et al. 2016). Quero-Garcia et al. (2009) found that mid-parent values were good predictions for progeny means for number of suckers, corm width, and dry matter content. Furthermore, the corm weight correlations were not significant and were remarkably lower than for corm dimensions. Soulard et al. (2016) found that the number of stolons, the number of suckers, fresh corm weight, and dry matter content were the most heritable traits. They also noted a moderate to high genetic gain for most heritable traits in early generation selections. Sugars, proteins and mineral content is negatively correlated to starch content in the corms, whereas starch and dry matter content is positively correlated (Lebot et al. 2011; Champagne et al. 2013). Cormel numbers and dry matter percentages have high heritability values in Indian taro germplasm. The number of cormels and dry matter percentages are positively correlated to tuber yield per plant. Weight of cormels per plant has a direct effect on tuber yield and is an important selection criterion to increase tuber yield per plant (Mukherjee et al. 2016).

1.4 Genotype by environmental interaction

Fox et al. (1997) defined genotype by environment interaction (GxE) as the differential expression of a genotype across environments. A genotype is the result of the action and interaction of those genes controlling a “character”. Environment refers to the “conditions” under which the plants grow and these environments consist of a combination of many biological, physical, and time factors which vary independently and

interact with each other. All these different factors have effects on the genotype to result in the specific phenotype that is observed (Romagosa and Fox 1993; Fox et al, 1997). GxE implies that even if all individual in a population were identical (with the same genotypes), they would not necessarily express their genetic potential in the same way with high variation in environmental conditions because genetic expression is a stochastic process.

Different types of GxE can be distinguished if the relative mean performance of each genotype in each environment is plotted against the environmental means (Figure 1.8). The two genotypes may react similarly to different environments (Figure 1.8a), or the two genotypes may react differently to the different environments. The ranking may stay the same (Figure 1,6b) or the ranking may change, crossover type, (Figure 1.8c) when two genotypes react differently to different environments. The type of GxE that has the biggest implication for plant breeders is the crossover type (Figure 1.8 c), which involves a change in rank order of the genotypes across environments. With crossover-interaction a genotype or variety recommended for one environment will not necessarily be that suited to another environment (Ramagosa and Fox 1993; Fox et al. 1997). The presence of GxE interactions implies that the relative behaviour of genotypes in a trial depends upon the particular environment in which they are being grown.

Becker (1981) cited by Fox et al. (1997) distinguishes two types of genetic stability: *biological or homeostatic stability,* which refers to genotypes that maintain a constant yield across environments; and *agronomic stability,* which refers to genotypes that yield according to the productive potential of the test environments. If a genotype exhibits

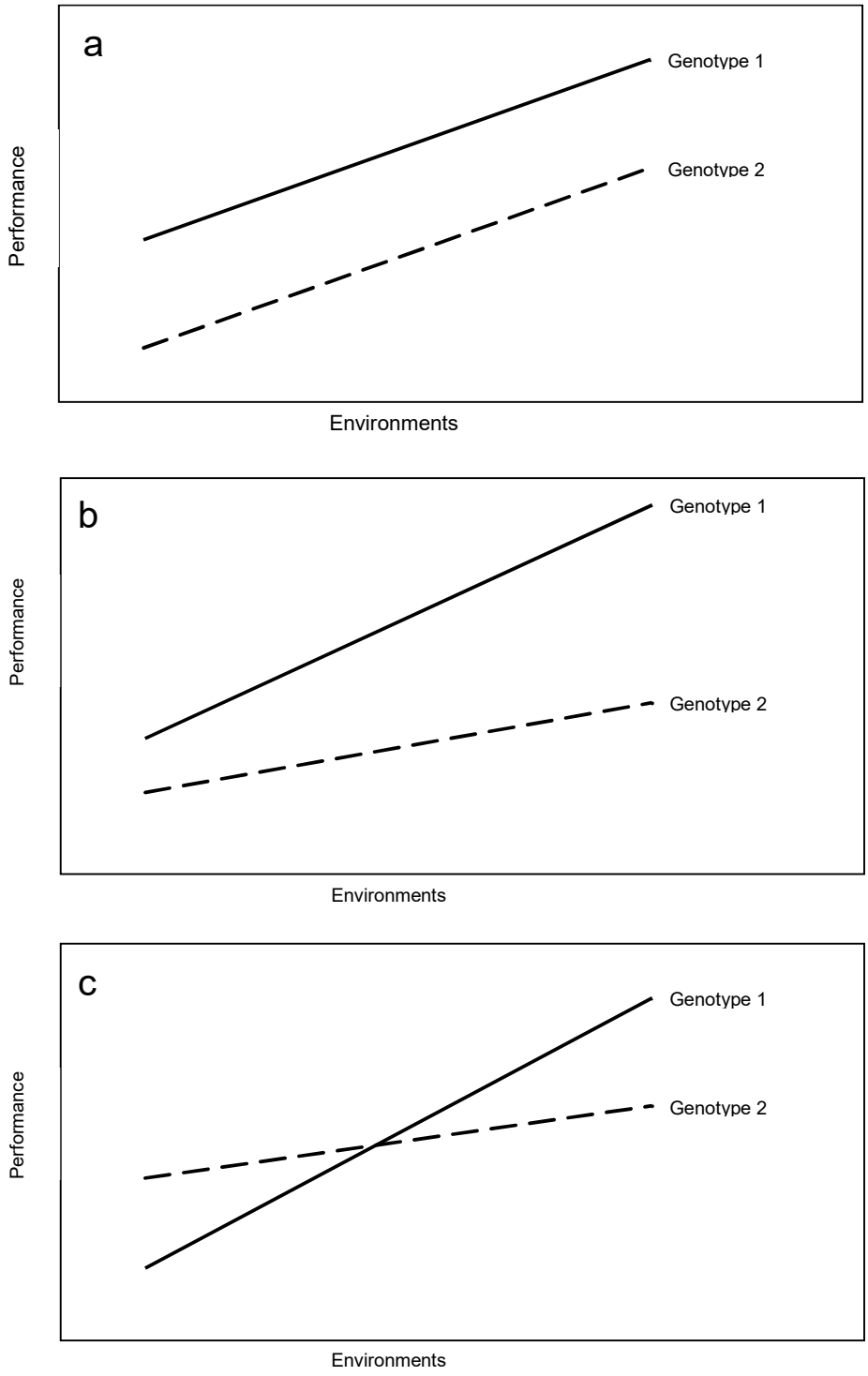

Figure 1.8: The performance of two hypothetical genotypes in two hypothetical environments, showing (a) no GxE interaction, (b) 'quantitative' GxE interaction (without reversal of ranks) and (c) "qualitative GxE interaction (with reversal of rank – crossover type) (Adapted from Romagosa and Fox 1993).

agronomic stability over a wide range of environments, the genotype is considered to have a general or wide adaptability. In contrast, if a genotype exhibits agronomic stability in a limited range of similar environments the genotype is considered to have a specific or narrow adaptability (Fox et al., 1997).

1.4.1 Statistical methods to measure GxE interaction

Various methods are used to analyse GxE interactions. These methods vary from analysis of variance (ANOVA) and regression, to non-parametric methods like pattern analysis and multivariate techniques (Ramagosa and Fox 1993; p 387).

1.4.1.1 Regression

Regression analysis is a statistical process for estimating the relationships among variables. Historically, regression was a popular statistical method to partition and analyse interaction (Gauch 1992). A model to determine genotype stability by simple linear regression was developed by Finlay and Wilkenson (1963). But even prior to Finlay and Wilkenson, Yates and Cochran proposed a similar method in 1938 (Gauch 1992). Ramagosa and Fox (1993) stated that the Finlay and Wilkinson regression is the most widely used (and, possibly misused) statistical technique in plant breeding.

Finlay and Wilkinson (1963) analysed the linear regression of the yield for each variety on the mean yield of all varieties for each site in each season. The mean yield of all the varieties at a specific site and season provides a numerical grading for sites and can be useful in evaluating the site's environment. The average yield of a large group of varieties can describe a complex natural environment without defining or analysing the interacting edaphic and seasonal factors (Finlay and Wilkinson 1963; Gauch 1992, p62).

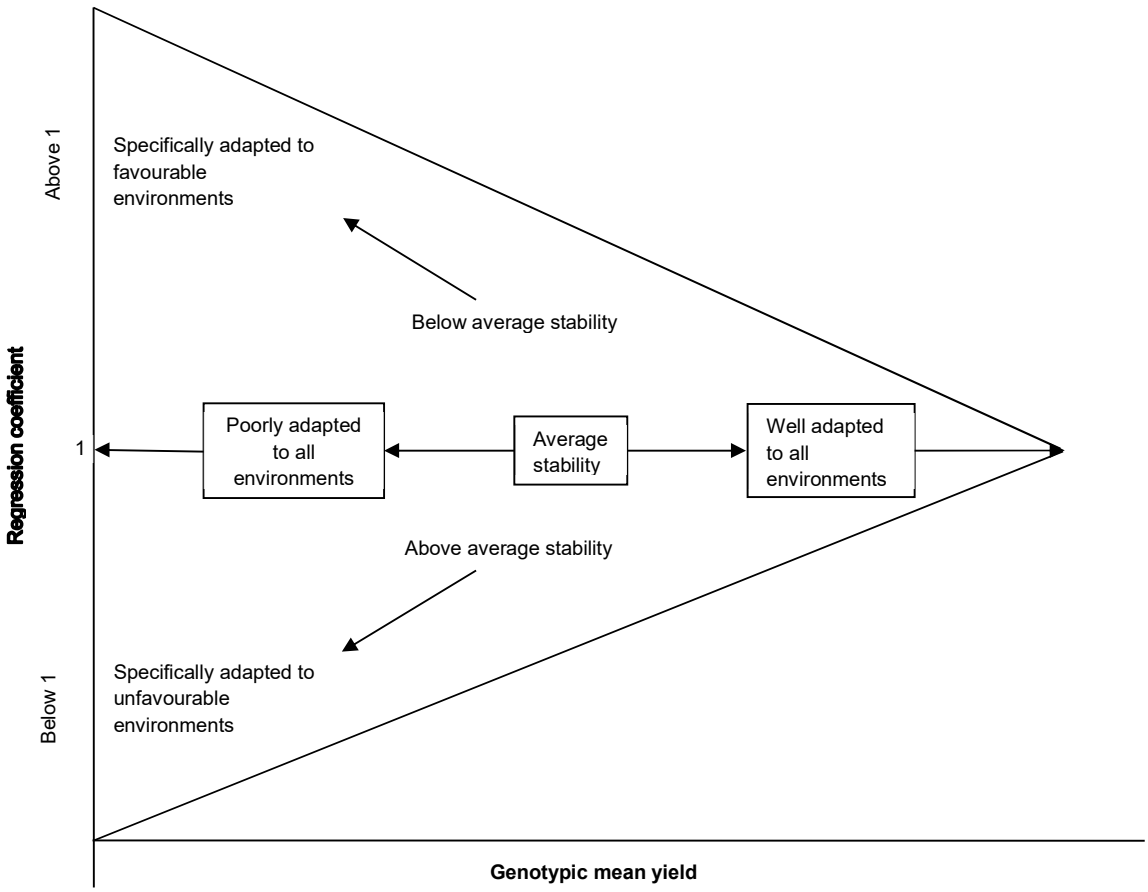

Figure 1.9: A generalized interpretation of the genotypic pattern obtained when genotypic regression coefficients are plotted against genotypic mean, adapted from Finlay and Wilkinson (1963).

The regression coefficients of a variety can be plotted against the variety's mean yield (Finlay and Wilkinson 1963). The population mean would have a regression coefficient of one. Regression coefficient for a variety in the order of 1.0 has an average stability over all environments. These varieties will perform consistently above or below the average for that environment, but their responses to changes in the environment will be the same. If the mean yield is below average these varieties are poorly adapted to all environments; if the mean yield is high, the variety is well adapted to all environments. Regression coefficients significantly larger than one (a very steep slope) are specifically adapted to high yielding/favourable environments and are sensitive to changes in environment. These varieties react very positively (increase in yield) for a small positive

change in environment. These varieties will have a low yield in unfavourable environments but will react positively to changes in environment and will yield above average in favourable environments. Regression coefficients significantly smaller than one (a flatter slope) are specifically adapted to low yielding/unfavourable environments. These varieties “resist” environmental changes and respond very little to large changes in environments; the varieties, as a result, have above average stability. They will have a good yield in unfavourable environments, but because they are less sensitive to environmental change, these varieties will still yield approximately the same at favourable environments where other cultivars might out-yield them. The interpretation of stability and adaptability by plotting the regression coefficient of a specific cultivar against the mean yield of that cultivar is summarized in Figure 1.9. Regression is effective to emphasize the trends of varietal responses in a range of environments (Finlay and Wilkinson 1963; Gauch 1992).

1.4.1.2 Analysis of variance

ANOVA offers an additive model for two-way data tables and analyse the differences between group means and their associated procedures (such as "variation" among and between groups). In the ANOVA setting, the observed variance in a particular variable is partitioned into components attributed to different sources of variation. The observed yield (Y_{ij}) of a given genotype “i” in environment “j” is portioned into (a) an additive model with three parameters, namely the grand mean μ, genotype deviation G_i, and environment deviation E_j, (b) the non-additive residuals or interaction GE_{ij} and error e_{ij} (Gauch, 1992, p 59).

The analysis of variance of a two-factor mixed model (fixed genotypes and random environment) expresses the observed (Y_{ij}) mean yield of the i^{th} genotype at the j^{th} environment as:

$$Y_{ij} = \mu + G_i + E_j + GE_{ij} + e_{ij}$$

Where μ is the general mean; G_i, E_j and GE_{ij} represent the effect of the genotype, environment, and the genotype environment interaction respectively; and e_{ij} is the average of the random errors associated with the r^{th} plot with the i^{th} genotype in the j^{th} environment. The most common analysis of variance is shown in Table 1.1 (Gauch 1992; Romagosa and Fox 1993). The mean describes the potential of an environment and the performance of a genotype when GxE is insignificant in a trial. However, the main effects should be interpreted with caution in the presence of significant GxE, and the nature of interaction needs to be investigated as the means can hide cases where certain genotypes perform very well or very poorly in specific environments. In the ANOVA, the size of the sums of squares of the relevant terms, and variance terms, are used to quantify the sources of variation (Ramagosa and Fox 1993).

Table 1.1: Two factor mixed model (fixed genotypes; random environment) analysis for *g* genotypes at *e* locations with *r* replicates per site (Ramagosa and Fox, 1993)

Source of variation	***Degrees of freedom***	***Mean Squares***	***Expected mean squares***	***F-ratio***
Total	*erg*-1			
Environ (E)	*e*-1	MS1	$\sigma^2_e + g\,\sigma^2_{R(E)} + rg\,\sigma^2_e$	MS1/MS2
Rep. E^2	e(*r*-1)	MS2	$\sigma^2_e + g\,\sigma^2_{R(E)}$	MS2/MS5
Genotype (G)	*g*-1	MS3	$\sigma^2_e + g\,\sigma^2_{GE} + er\Phi^2_E$	MS3/MS4
G X E	(*e*-1)(*g*-1)	MS4	$\sigma^2_e + g\,\sigma^2_{GE}$	MS4/MS5
Error	*e*(*g*-1)(*r*-1)	MS5	σ^2_e	

g – genotype*; e* – environment*; r* – replicates; σ^2 - population variance; Φ^2 –Genotypic variance

Small means describe the potential of an environment and the performance of genotypes adequately if there is no significant interaction between the genotypes and the environment. However, if the interaction is significant, the means may mask genotypes that perform particularly well or poorly in a subset of the environments (Ramagosa and Fox 1993).

1.4.1.3 Principal Component Analysis (PCA)

Principal component analysis (PCA) offers a multiplicative model for analysis in contrast to the additive model of ANOVA (Gauch 1992, p 69). In an additive model the effects of individual factors are differentiated and added together to model the data, whereas in a multiplicative model the joint effect of two or more causes is the product of their effects, if they were acting alone. ANOVA analysis results in only one set of genotype and environmental deviations, whereas PCA can give several sets of parameters (axes), PC1, PC2, PC3……PCn. The PCA offers a series of models that result in one full model (Gauch 1992, p 69-70).

PCA reduces the dimensionality of multivariate data and makes it possible to visualise it in fewer dimensions (normally 2) in a series of biplots (Gauch 1992 p 71). The PCA biplots have two types of points, genotype and environment; interpretation of the biplot involves analysing the relationships amongst points of the same kind and the relationship between points of different kinds. Amongst points of the same type, points that are close to each other are similar and points that are far apart are dissimilar. When interpreting different kinds of points, a genotype's score can be multiplied with the environment's score to give the PCA models' expected value for that genotype in that environment. Scores near zero represent genotypes or environments with small variations (for the specific characteristic), whereas genotypes or environments with large values (positive or negative) have large variations. Expected values for a characteristic far above the grand mean involve genotypes with large positive scores of the same sign, while relatively small values for the characteristic involving large scores of opposite signs. Genotypes with large positive scores grow very well in environments with a positive score, but especially poorly in environments with a large negative score. The opposite is also true; genotypes with a large negative score grow well in environments with a negative score. Thus, the distribution of points on the PCA biplot can be used to interpret

and explain responses and interactions, and can be complimented by additional knowledge of the environments and genotypes (Gauch 1992).

1.4.1.4 Additive main effects and multiplicative interaction (AMMI)

The additive main effects and multiplicative interaction, or AMMI, method combines the standard ANOVA for the genotype and environment main effects with PCA. The AMMI model separates the additive variance from the multiplicative (interaction) variance and applies PCA to the interaction portion from the ANOVA analysis. The ANOVA partitions the total variation into three orthogonal sources, namely genotype; environment and genotype environment interactions. The AMMI then uses a PCA to partition the genotype environment interactions into several orthogonal axes (interaction principle component axes) that account more effectively for the interaction patterns (Shaffi et al 1992; Gauch and Zobel 1996; p 85; Hill et al., 1997). Hill et al (1997) noted that the AMMI strips away the additive effects of genotype and environment from the two-way genotype-environment table and conducts a PCA on the residual.

The AMMI analysis generates a series of models, designated as AMMI0, AMMI1, AMM2, AMMI3…. AMMIF depending on the number of axes retained. AMMI0 fits only additive main effects of genotypes and environments, but retains no interaction principal component axes (IPCA). AMMI1 fits the additive effects from AMMI0 plus the interactions associated with the first principal component axis (IPCA1). AMMI2 fits the interaction associated with IPCA2 and so on up to AMMIF, the full model that retains all the axes (Hill et al., 1997).

The AMMI results can be plotted in a biplot that shows main and interaction for genotypes and environments. The AMMI1 places the genotype and environment means on the X axis and the respective eigenvectors on the Y axis. Genotypes and environments that fall in a vertical line have similar means, and genotypes and

environments that fall on a horizontal line have similar interaction patterns. Genotypes or environments with a large first principal component axis score have high interactions, and those with values close to zero have small interactions. Polygons may be applied to AMMI2 to show which genotype is the most successful in each environment (Gauch and Zobel 1996; p 89, 90; Hill et al., 1997).

The AMMI equation is expressed thus:

$Y_{ger} = \mu + \alpha_g + \beta_e + \Sigma_n \lambda_n Y_{gn} \sigma_{en} + \rho_{ge} + \varepsilon_{ger}$

Where:

Y_{ger} = observed yield or phenotype of genotype g in environment e for replicate r.

Additive parameters:

μ = grand mean

α_g = deviation of the genotype

β_e =deviation of the environment

Multiplicative parameters:

λ_n = singular value for the interaction principal component axis (IPCA) n

Y_{gn} = genotype Eigen vector for axis n

σ_{en} = environment Eigen vector

ρ_{ge} = residuals

ε_{ger} = error (Gauch and Zobel 1996; p86)

The AMMI method is used for three main purposes - model diagnosis, to clarify GxE interactions, and to improve accuracy of estimates. AMMI is more appropriate in the initial statistical analysis of yield trials, because it offers an analytical tool for diagnosing models as subcases when these are better for a particular data set. AMMI is also used to clarify GxE interactions. AMMI plots summarize patterns and relationships of genotypes and environments. AMMI can also improve the accuracy of yield estimates that are equivalent to increasing the number of replicates, thus reducing the costs of trials by reducing the number of replications, or creating the opportunity to include more varieties in the experiment. Additionally, it will improve the efficiency in selecting the best genotypes (Crossa 1990).

AMMI has proven useful for understanding complex GxE interactions, as the results can be plotted in a very informative biplot which shows both main and interaction effects for both genotypes and environments. Additionally, AMMI can partition the data into a pattern rich model and discard noise residual to gain accuracy (Gauch and Zobel 1996; p85).

1.4.2 Genotype x environment interaction in *Colocasia esculenta*

Different taro types are found globally. Some types are adapted to paddy conditions, others to upland conditions, while some even tolerate relatively long periods of drought. Furthermore, some types are only adapted to coastal areas or higher altitudes (Lebot 2009).

Ivancic and Lebot (2000) found that none of the more than 2000 genotypes tested in paddy conditions in Papua New Guinea were adapted to paddy growing, they noted that it might be an indication of a narrow range of environmental adaptability. Okpul (2005) tested seven taro elite lines, and a highly preferred control cultivar, 'Numkoi', in seven diverse agro-ecological environments. There were significant differences in yield among genotypes at six sites, and significant GxE interaction (Okpul 2005)

1.5 Justification and study objectives

Only taro landraces are used in South Africa and in Africa. No local genetically improved material exists and taro genetically improved germplasm is imported from the various breeding programs and the Secretariat of the Pacific Community (Suva, Fiji), that host the largest aroid germplasm collection in the world. Literature indicate that the diversity of taro is low in African countries. This also seems to be the case in South Arica superficially; however, no information is available for South Africa. Worldwide, very little is known about the influence of the environment on the performance of specific

landraces. The study intend to highlight certain aspects of the genetic improvement of taro in South Africa. It will attempt to establish the genetic diversity of taro in South Africa. The study will also attempt to generate diversity by means of hand pollinations. Finally, the study will attempt to determine the influence of the environment on taro landraces.

The specific objectives of the study are:

- To determine the genetic diversity in the ARC taro germplasm collection using agro-morphological characteristics and microsatellite markers.
- To determine if it is possibility to breed with local taro germplasm.
- To determine the effect of four different environments (Roodeplaat, Umbumbulu, Owen Sithole College of Agriculture and Nelspruit) on ten agro-morphological characteristics of 29 taro landraces.

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Chapter 2: Genetic diversity of *Colocasia esculenta* in South Africa

Abstract

Amadumbe (*Colocasia esculenta*), better known as taro, is a traditional root crop in coastal areas of South Africa. Taro is showing potential for commercialisation. However, very little is known about the introduction and movement of taro in South Africa. More information on the genetic diversity of taro is necessary before any genetic improvement can be attempted. This study investigated the diversity within the Agriculture Research Council (ARC) germplasm collection using agromorphological descriptors and simple sequence repeat (SSR) markers,

Taro germplasm was collected in South Africa in order to build up a representative collection with 77 local accessions as well as foreign accessions. Germplasm was also imported from Nigeria and Vanuatu. The South African taro germplasm, as well as selected imported germplasm, was characterised using key agro-morphological descriptors as proposed by Singh et al (2008). Theas well as simple sequence repeats (SSR) developed by Mace and Godwin (2002). Dendrograms were constructed using UPGMA cluster analysis.

Very little variation was observed between the South African accessions using agro-morphological descriptors. No variations were observed for eight of the 30 agro-morphological characteristics. These eight characteristics are leaf blade colour variegation, predominant position of leaf lamina surface, leaf main vein colour, leaf vein pattern, petiole basal ring colour, type of leaf blade variegation, colour of leaf blade variegation). The 86 accessions were grouped into three clusters. The three clusters contained 39, 20 and 27 accessions respectively.

SSR primers revealed polymorphisms for the South African germplasm. Primer Uq 84 was highly polymorphic. The accessions grouped into five clusters with 33, 6, 5, 41 and 7 accessions in each of the clusters. All the dasheen type accessions clustered together.

A higher level of genetic diversity in the South African germplasm was observed when molecular analysis was compared to with morphological characterisation. No correlation was detected between the different clusters and geographic distribution, since accessions from the same locality did not always cluster together, or conversely, accessions collected at different sites were grouped together. There was also no clear correlation between the clustering based on agro-morphology and SSRs. Thus in order to obtain more complete characterisation, both molecular and morphological data should be used. Although the results indicated that there is more diversity present in the local germplasm than expected, the genetic base is still rather narrow, as is the case in other African countries.

2.1 Introduction

The existing variation, due to genetic differences, within a population or species is called genetic diversity. Genetic diversity is important for the survival and adaptability of species. Species with high genetic diversity will produce a wider range of offspring. Some of the offspring will better adapt than others. Genetic diversity, therefore, facilitates populations or species adaptation to changing environments (Devi 2012; NBII 2017). Genetic diversity within and between populations or species can be assessed using various parameters and methods such as;

- agro-morphological (growth habit, stolon formation, plant height, shape, colour and orientation of lamina, maturity, shape and weight of corms and cormels, corm and cormel yield, flesh colour and edibility of tubers, resistance against leaf blight etc.),
- biochemical (protein expression profiles and isozymes) and

- molecular markers (DNA markers e.g. RAPD, AFLP, SSRs etc.) (Nybom 2004; Devi 2012).

Taro exhibits a wide array of agro-morphological variation. Okpul et al. (2004) and Singh et al. (2008) have developed concise descriptor lists for taro. The level of morphological variability reported by authors vary. Okpul et al. (2004), Lebot et al. (2000) and Godwin et al. (2001) observed high morphological variation in areas where there is natural sexual recombination as well as exchange of germplasm.

Various DNA markers were used to determine genetic diversity in taro (Lebot, 2009). These include random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLP). Simple sequence repeats (SSR) or microsatellite polymorphisms have a higher mutation rate than other DNA areas leading to high genetic diversity. Mace and Godwin (2002), Noyer et al. (2004), Singh et al. (2008), Hu et al. (2009), Sardos et al. (2011), Singh et al. (2011), Lu et al. (2011), You et al. (2014) and Chaïr et al. (2016) have used SSRs to study genetic diversity in taro. They proved to be a valuable tool for the identification of duplicates (Mace and Godwin, 2002). The SSRs did not reveal any clear geographical structure (Mace and Godwin 2002; Quero-Garcia et al. 2006); however, Sardos et al. (2011) did found a degree of correlation between geographical and present social and genetic diversity and SSRs diversity. SSRs were able to discriminate between diploid and tetraploid germplasm (Chaïr et al. 2016).

Higher levels of genetic diversity are reported for taro originating from South East Asia than those originating from Africa and the Pacific region (Safo Kantaka 2004; Lebot 2009; Paul et al. 2011; Orji and Ogbona 2015; Chaïr et al. 2016). The highest allelic diversity is observed in South-East Asia (Indonesia, Malaysia, Thailand and Vietnam, Bangladesh, Japan and New Guinea) (Isshiki et al. 1998; Lebot et al. 2000; Safo

Kantaka 2004; Lebot 2009 and Paul et al. 2011). The Pacific Countries (the Philippines, Papua New Guinea Vanuatu) (Lebot et al. 2000; Safo Kantaka 2004; Lebot 2009 and Paul et al. 2011) and Africa (Safo Kantaka 2004; Fujimoto 2009; Lebot 2009; and Mwenye et al. 2016) appear to have limited allelic diversity. The highest level of diversity were observed in the centre of origin for taro and in areas where natural sexual recombination occurs (Safo Kantaka 2004; Lebot 2009).

No genetically improved taro germplasm occurs in South Arica. However, the farmers are able to distinguish between different landraces. No farmer was able to distinguish between more than six landraces (Mare 2006). Mabhaudhi and Modi (2013) distinguished between three taro landraces using agro-morphological characteristics and SSRs. Chaïr et al. (2016) are of the opinion that South African taro shared a lineage with Japanese taro. Mare (2006) noted that there might only be four taro landraces in the study area in KwaZulu-Natal. This study attempted to characterise the South African taro collections based on morphological descriptors and SSRs.

2.2 Material and Methods

2.2.1 ARC Roodeplaat-germplasm collection

The taro germplasm collection of Agricultural Research Council (ARC) consists of 77 local landraces, collected in KwaZulu-Natal, Mpumalanga and Gauteng, five accessions from Nigeria, eight accessions from Vanuatu and one from South East Asia. The name of the location was assigned as accession/landrace name if no name already exist for the accession/landrace. Sixty eight seedlings from a mixture of seed from Vanuatu were also part of the collection. The whole ARC taro germplasm collection is presented in Appendix 1, however, not all these accessions were included in the analysis. The geographic coordinates of the collection localities for all South African accessions were plotted with DIVA (Diversity analysis software) (Figure 2.1) (Hijmans 2004). All accessions are maintained in a “pan and fan” glasshouse, without any climate control,

at the ARC. Sixty accessions were selected form the whole collection and multiplied in the field.

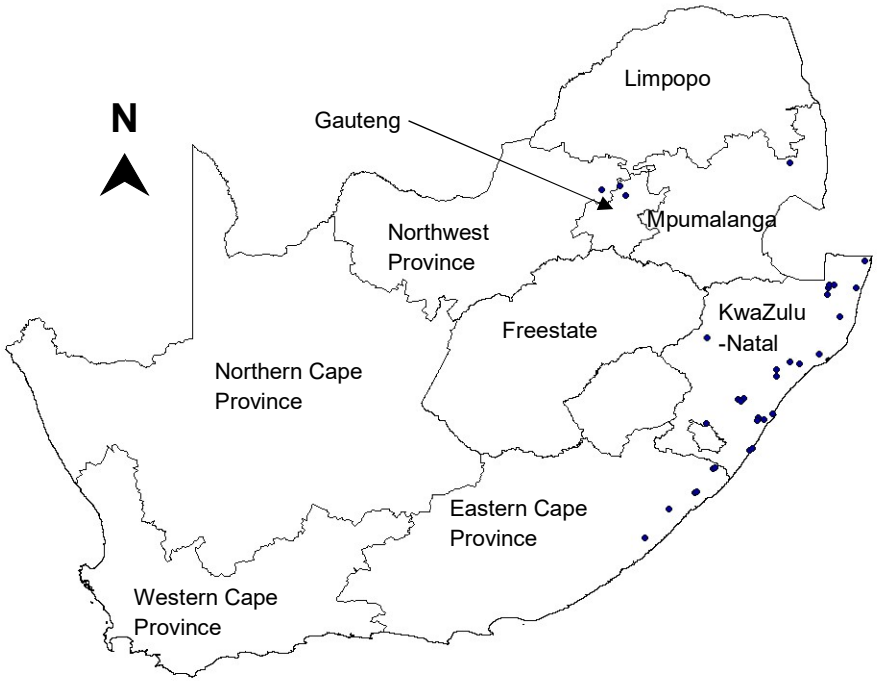

Figure 2.1: Distribution of collection localities for the South African *Colocasia esculenta* accessions. Map drawn with DIVA (Hijmans *et al.* 2004).

2.2.2 Genetic diversity studies

Genetic diversity studies were done using morphological descriptors (Sing *et al.* 2008) and microsatellite markers.

2.2.2.1 Morphological descriptors

All the accessions in the ARC genebank were scored according to the subset of descriptors used by Singh *et al.* (2008). The list contain 10 quantitative and 20 qualitative characteristics. The data sheet used for scoring is presented in Appendix 2. Data matrixes were analysed using the Phylogenetic Analysis Parsimony (PAUP) and EXCELSTAT to obtain a better understanding of the relationship between the different

accessions. Cladograms are constructed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm. The Dice dissimilarity index used was [2a/(2a + b + c))]. This method, first described by Sneath and Sokal (1973), used the concept of minimal dissimilarity between two "neighboring" points and an ultrametric distance (Sokal and Michener 1958; Sneath and Sokal 1973).

2.2.2.2 SSR markers:

Genomic DNA was isolated using the CTAB method (Edwards, Johnson and Thompson 1991). Two leaf discs were collected in a microfuge tube by punching the discs directly into the tube, using the lid as a punch. A pinch of Carborundum was added and the leaf material grounded thoroughly in the microfuge tube with a clean glass grinder. A 400 ml warm (60°C) CTAB buffer was added to the grounded leaf mixture and incubated at 60°C (waterbath) for 30 minutes. Equal volume of chloroform:isoamylalcohol (24:1) was then added and mixed (5 minutes) by inverting the tube several times. The mixture was then centrifuged at 10 000 g for 10 minutes (Allegra X-22R, Bench Top, Beckman). The supernatant was then carefully transferred into a clean microfuge tube and 0.6 volumes of ice-cold isopropanol added to the supernatant. The mixture was gently mixed by inversion and left at –20°C for 30 minutes. The mixture was centrifuged at 10 000 x g for 10 minutes and decanted to drain the isopropanol and retain the DNA pellet. The pellet was washed using 70% ethanol followed by quick centrifugation to help the DNA pellet seat at the bottom of the tube and drain the ethanol. The pellets were left to air dry. The resulting pellets were re-suspended in 50 µl TE buffer. Concentration of the genomic DNA was determined by fluorometer with a SEQUOIA-TURNER Model 450 digital fluorometer. DNA concentration was adjusted at 10 ng/µl for all samples.

Six pairs SSR primer sets with forward and reverse sequence were chosen from those published by Mace and Gordon (2002). The SSR primers used were Uq 55; Uq 73; Uq 84; Uq 88, Uq 97; Uq 110; and Uq 115 . Polymerase chain reactions were run

according to Mace and Gordon (2002) and Singh *et al* (2008). All reactions were done in labelled autoclaved 0.2 ml microfuge tubes. Reaction cocktails were prepared as specified in Table 2.1. The aliquot of reaction cocktail was made and transferred (7.5 µl/tube) into labelled reaction tubes and 5µl of the template DNA solution added to the reaction cocktail.

Table 2.1: Reaction cocktail for SSR reactions

Reagents	**[Stock]**	**[Final]**	**Amount 1 x reaction (µℓ)**
dH_2O			2.05
10 x Buffer	10 x	1 x	1.25
$MgCl_2$	25 mM	3.5 mM	1.75
Dntp	2.5 mM	0.4 mM	2.0
Primer 1	20 µM	0.24 µM	0.15
Primer 2	20 µM	0.24 µM	0.15
TaKaRa TAQ	5 U/µl	0.75 U	0.15
Template DNA	10 ng/µl	50 ng	5.0
TOTAL			12.5

Amplification was done in a PTC 100 thermocycler and programmed to one 60 sec cycle at 95°C, followed by 35 cycles of 30 sec at 95°C to denature template DNA, 45 sec 55°C to anneal primers to template DNA and 120 sec at 72°C for elongation and amplification. A final elongation cycle of 420 sec at 72°C was applied.

Amplification products were separated with polyacrylamide gel electrophoresis and stained with silver staining. Resulting bands were scored in a binary matrix noting the absence (0) or presence (1) of specific bands. These bands were treated as genetic loci.

The data was analysed using the Phylogenetic Analysis Parsimony (PAUP) phylogenetic programme to obtain a better understanding of the relationship between the different accessions. Cladograms were constructed using the Unweighted Pair Group Method with Arithmetic mean (UPGMA) algorithm.

2.3 Results

2.3.1 Morphological diversity

South African accessions all look identical except for the leaf blade margin colour, petiole junction pattern and colour, colour of basal third of the petiole colour and the presence and colour of the petiole stripe. Different corm shapes were observed in informal markets in South Africa, however, germplasm from these landraces was not included in the study due to the low availability of material. However, the seedlings from Vanuatu exhibited a wide range of diversity (Figure 2.2).

No morphological differences were observed for eight characteristics, namely (1) leaf blade colour variegation, (2) predominant position of leaf lamina surface, (3) leaf main vein colour, (4) leaf vein pattern, (5) petiole basal ring colour, (6) type of leaf blade variegation, (7) colour of leaf blade variegation, and (8) leaf blade colour. Only one accession, 71 (Dumbekele collected in the Valley of a Thousand Hills in KwaZulu-Natal) flowered naturally. The corm cortex and flesh colour was either cream or white. However, there was an anecdotal report of purple fleshed taro grown in the Inanda area, close to Durban. The corm fibre colours were cream, white or purple. None of the South African landraces collected had stolons.

All the accessions, except two, had a white corm cortex and flesh (97.3%). Only two (2.33%) accessions had cream corm cortex and flesh. The colour of the corm fibres varied from white (70.9%) to light yellow (25.6%), yellow (1.2%) to purple brown (2.3%). The predominant corm shape was elliptical (60.5%), followed by round (33, 7%) and

elongated corms (5.8%). Petiole junction colouration was absent in 8.1% of the accessions. The petiole junction colouration was mostly purple (77.9%) or green (14%) if present. The petiole junction pattern was generally very small (84.9%) in the South African germplasm. The colour of the lower part of the petiole was one of the most variable characteristics in the South African germplasm. The lower part of the petiole was purplish brown (65.1%), green (27.5%), light green (3.5%) purple (1.2%) or almost white (1.2%). A petiole stripe was present in 54.7% of the accessions. The petiole stripe was always purple if it was present. The petiole top colour was predominantly purple (90.7%) or green (9.3%). Only one accession (1.2%) flowered naturally.

Light green

Brown green

Purple

Variation in petiole base colour

Few suckers

Moderate nymber of suckers

Many suckers

Variance in amount of suckers

No stolons

Short stolons

Long stolons

Variation in length and number of stolons

Green

Green brown

Purple

Variation of variegation in petioles

Green shoulder

Light purple shoulder

Dark purple shoulder

Variation in petiole shoulder colour

All veins purpel

All veins green

Primary veins purple

Variation in colour of main veins

No colour in petiole junction | Large petiole junction | Petiole juction colour extend into the veins

Variation in colour and pattern of petiole junction on adaxial side of the leaf

Purple variegated | Green | Purple

Variation in leaf blade colour
r

No waviness | Medium waviness | Very wavy

Variation in waviness of the leaf blade margin

Figure 2.2: Vanuatu seedling accessions germinated at ARC VOP to illustrate the variability in certain characteristics (WS Jansen van Rensburg).

Agglomerative hierarchical clustering (AHC) revealed that the 86 accessions were grouped into three clusters (Figure 2.3 and Table 2.2). Cluster 1 contained 39 accessions (Table 2.2). It included accessions collected at Hluhluwe (all except 1),

Maphumulo, Warwick market, Willowvale, Creighton, Isiphingo (3), Umbumbulu (2), Pietermaritzburg, Nelspruit, Manguzi, Mtwalume, Makatini and Pieter Maritz (commercial farmer). The accessions in this group were collected in Mpumalanga, KwaZulu-Natal (North central and South) and Eastern Cape. The presence of a purple petiole stripe was unique to this group. Coco India, an accession from Nigeria, was also included into this cluster.

Cluster 2 included 20 accessions (Table 2.2). Accessions were collected at Hluhluwe, Maphumulo, Mkuze (all accessions collected at Mkuze), Makatini, Lusikisiki, Umbumbulu, Jozini, Empangeni and Pietermaritzburg (from Prof. Albert Modi, UKZN). Ukpong and Nigeria, two accessions from Nigeria, were also included in this cluster. All the accessions, except for Ukpong and Nigeria, were collected in Northern and Central KwaZulu-Natal and Eastern Cape. None of the accessions in this group had a purple-brown lower petiole colour.

Cluster 3 consisted of 27 accessions (Table 2.2). The accessions were collected in Jozini, Lusikisiki, Eshowe (3), Brits, Tshwane, Pietermaritzburg, Soshanguve, Mtwalume, Maphumulo, and Dumkehle (Valley of a Thousand Hills), Ghana and an accession from Nigeria that was also included belonged to this cluster. All the accessions, excluding Ghana, were collected in the Northern, Central and Southern KwaZulu-Natal and Gauteng. The lower petiole colour of all accessions in this group was purple-brown. There was no obvious correlation between the origin of the accessions and the clusters. The most important characteristics seemed to be the colouration of the petiole.

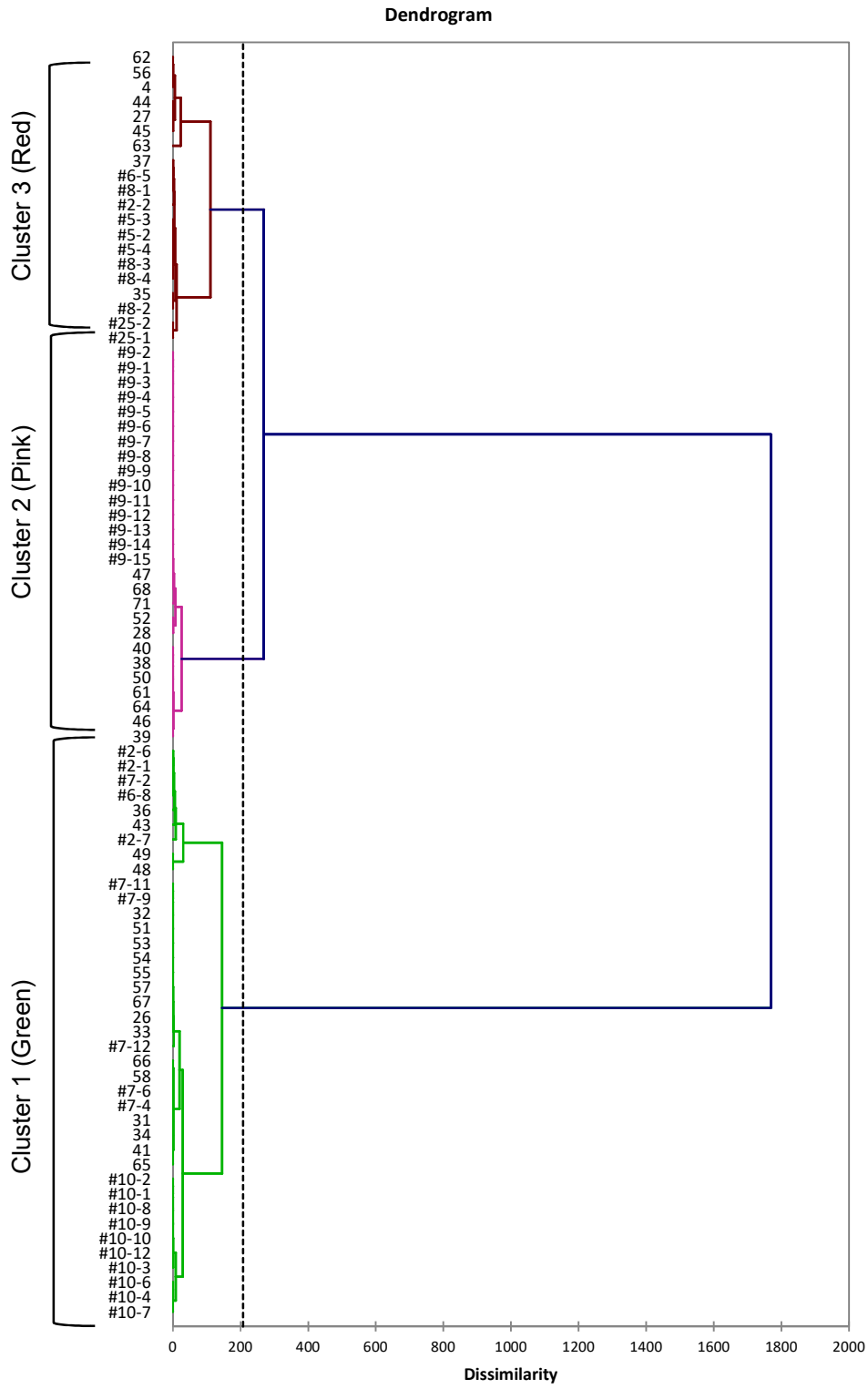

Figure 2.3: Agglomerative hierarchical clustering (AHC) of 86 South African taro accessions based on agro-morphological descriptors. The Euclidean coefficient of dissimilarity between accessions is indicated on the X axis. The accessions cluster in three clusters with a threshold at 200.

Table 2.2: South African taro accessions grouped into three clusters. The central accession (centroid) for each cluster is in red bold face.

Cluster	Number of accessions	Minimum distance to cluster centroid	Average distance to cluster centroid	Maximum distance to cluster centroid	Accessions
1 Red in Fig ,2,3	39	0.960084	2.304408	5.722087	#2-1, #2-6, #2-7, #6-8, #7-2, #7-4, #7-6, #7-9, #7-11, #7-12, #10-1, #10-2, #10-3, #10-4, #10-6, #10-7, #10-8, #10-9, #10-10, #10-12, **26**, 31, 32, 33, 34, 36, 41, 43, 48, 49, 51, 53, 54, 55, 57, 58, 65, 66, 67
2 Pink in Fig 2.3	20	1.600781	2.75016	5.758689	#2-2, 4, #5-2, #5-3, #5-4, #6-5, #8-1, #8-2, #8-3, #8-4, **#25-1**, #25-2, 27, 35, 37, 44, 45, 56, 62, 63
3 Green in Fig 2.3	27	0.417386	0.960735	2.507055	**#9-1**, #9-2, #9-3, #9-4, #9-5, #9-6, #9-7, #9-8, #9-9, #9-10, #9-11, #9-12, #9-13, #9-14, #9-15, 28, 38, 39, 40, 46, 47, 50, 52, 61, 64, 68, 71

Clusters 2 and 3 are grouped closer together compared with cluster one (Table 2.2 and Figure 2.3). The distance between cluster 2 and 3 is only 5.477 units (Table 2.2) whereas the distance between cluster 1 and 2 and 1 and 3 is 10.149 and 9.110 units (Table 2.3) respectively.

The multivariate analysis revealed that the first five principal components (PC1 to PC5) gave Eigen-values higher than 1.0 and cumulatively accounted for 85.387% of the total variation (Table 2.4).

Table 2.3: Distances between the central accession in each of the three clusters.

	1 (26)	2 (#25-1)	3 (#9-1)
1 (26)	0	10.149	9.110
2 (#25-1)	10.149	0	5.477
3 (#9-1)	9.110	5.477	0

Table 2.4: Variation accounted for by each principal (PC) component in the principal component analysis.

	PC1	PC2	PC3	PC4	PC5[1]
Eigenvalue	2.783	2.072	1.882	1.654	1.001
Variability (%)	25.302	18.833	17.112	15.036	9.103
Cumulative %	25.302	44.136	61.247	76.284	85.387

[1] The first five PCs have eigenvalues of more than one, contributing significantly to the total variation

Table 2.5: The correlation coefficients[1] of each trait/characteristic with respect to each principal component.

Traits	Code	PC1	PC2	PC3	PC4	PC5
Corm cortex colour	CCC	0.214	**0.881**	0.175	-0.380	0.059
Corm flesh colour	CFL	0.214	**0.881**	0.175	-0.380	0.059
Corm fibre colour	CFI	0.314	0.369	0.126	**0.730**	0.028
Corm shape	COS	0.352	0.265	-0.312	0.443	-0.282
Petiole junction colour	PJC	**0.809**	-0.085	-0.330	-0.131	-0.177
Petiole junction pattern	PJP	**0.869**	-0.045	-0.030	0.363	0.084
Petiole lower colour	PLC	0.414	**-0.405**	-0.071	**-0.599**	0.183
Presence of petiole stripe	PPS	0.193	-0.180	**0.906**	0.108	0.041
Petiole stripe colour	PSC	0.411	-0.251	**0.822**	-0.006	0.006
Petiole top colour	PTC	**0.810**	-0.215	-0.192	-0.287	-0.122
Flower formation	FFT	0.164	0.007	-0.242	0.182	**0.909**

[1] The correlation coefficients are an indication of the contribution of each trait to the specific PC. Traits that contribute significantly to the variation explained by a PC are presented in boldface.

The association of considered traits with specific PC's are presented in Table 2.4 and Table 2.5. Variation in PC1 was mainly associated with petiole junction colour, pattern

and the colour of the petiole top, and contributed to 25.3% of the variation. Variation in PC2 was associated with the corm cortex and flesh colour, the colour of the lower part of the petiole, and contributed to 18.3% of the variation. Variation in PC3 was associated with the presence and colour of the petiole stripe (17.1% of the variation), while PC4 was associated with corm fibre colour and the colour of the lower part of the petiole (15.0% of the variation). Variation in PC5 is associated with flowering and contributes 9.1% of the variation.

2.3.2 Molecular analysis

The SSR primers used, namely; Uq 55, Uq 73, Uq 84, Uq 88, Uq 97, Uq 110 and Uq 115 (Mace and Godwin, 2002), revealed polymorphisms for the South African germplasm. Four primer pairs, namely; Uq 55; Uq 73; Uq 84 and Uq 88 gave the best results. Primer Uq 84 was the most useful (highly polymorphic) because of the higher number of alleles detected from it and the polymorphisms in alleles which made it easy to score.

The data matrix was analysed and the cladogram (Figure 2.4) revealed that the accessions grouped into five clusters based on their dissimilarity. Table 2.1 lists the accessions within each cluster. The central object for cluster 1 is JoziniZulu7, cluster 2 is Maphumulo4, cluster 3 is Makatini RS48, cluster 4 is MaphumuloLG2 and cluster 5 is Ngqeleni30. Table 2.7 provides the distance between the centre accessions of each cluster. These distances vary from 1 between cluster 1 and 4, and 4.472 between cluster 2 and 5. This can also be seen in the cladogram (Figure 2.4 and Table 2.7).

Cluster 1 consisted of 33 accessions, JoziniZulu7 is the centre accession. Within cluster 1, Lusikisiki 28, Isipinho 32 and 33, Umbumbulu 35 and 37, Pietermaritzburg 36, Eshowe 39, Mkuz 5-4, MaphumuloLG 5 and JoziniZulu 1, 2, 3, 4, 5 and 6 seem to be identical. JoziniZulu 7 and 8, Shoshanguve 52, Umumbulu 53 and 54, AModi 55 and 56, Cocoindia

58, Ghana 61, Mtwalume 64 and 65, MakatiniMpondo 66, MakatiniRound 67 and Maphumulo07 68 are apparently identical.

Table 2.6: Accessions within the respective five clusters formed by SSR analysis

Cluster	Number of accessions	Accessions within the respective clusters
1	33	Hluhluwe1,Isipingo32,Umbumbulu37,Mkuze3,Lusikisiki28,Isipinho32, Isipinho33,Umbumbulu35,Pietermaritzburg36,Umbumbulu37,Eshowe39, Mkuz5 4,MaphumuloLG5,JoziniZulu1,JoziniZulu2,JoziniZulu3,JoziniZulu4, JoziniZulu5,JoziniZulu6,JoziniZulu7,JoziniZulu8,Shoshanguve52,Umumbulu53, Umumbulu54,AModi55,AModi56,Cocoindia58,Ghana61,Mtwalume64, Mtwalume65,MakatiniMpondo66,MakatiniRound67,Maphumulo0768
2	6	Pietermaritzburg56, Maphumulo4, Lusikisiki27, Jozini44, Empangeni45, MakatiniMpondo2
3	5	Nigeria63, Black Knight70, Makatini RS48, MakatiniD649, Dumkehle71
4	41	Hluhluwe2,Hluhluwe6,Hluhluwe7,MaphumuloLG2,MaphumuloLG3, MaphumuloLG6,MaphumuloLG8,Warwick2,Warwick4,Warwick6,Warwick9, Warwick11,Warwick12,Mkuze1,Mkuze2,Mkuze4,Mbazwana1,Mbazwana2, Mbazwana3,Mbazwana4,Mbazwana6,Mbazwana7,Mbazwana8,Mbazwana9, Mbazwana10,Mbazwana12,Hiberdene2,MakatiniMpondo11,Wollowvale26, JoziniZulu9,JoziniZulu10,JoziniZulu11,JoziniZulu12,JoziniZulu13,JoziniZulu14, JoziniZulu15,MakatiniMpondo1,Brits46,VilieriaFV47,PNatalAgricShow50, Shoshanguve51
5	7	Ngqeleni30, Creighton31, Isipinho34, Eshowe38, Eshowe40, Nelspruit41, Mangozi43

Cluster 2 consist of six accessions with Maphumulo4 the centre accession. Cluster 3 consist of five accessions with Makatini RS48 the centre accession. Cluster 5 consist of seven accessions with Ngqeleni 30 the centre accession.

Table 2.7: Distances between the central objects for the five clusters formed by SSR analysis. The central accession of each cluster is presented in brackets in the heading row.

	Cluster 1 (JoziniZulu7)	Cluster 2 (Maphumulo4)	Cluster 3 (Makatini RS48)	Cluster 4 (MaphumuloLG2)	Cluster 5 (Ngqeleni30)
Cluster 1	0	4.359	3.162	1.000	2.236
Cluster 2	4.359	0	3.873	4.243	4.472
Cluster 3	3.162	3.873	0	3.000	3.606
Cluster 4	1.000	4.243	3.000	0	2.000
Cluster 5	2.236	4.472	3.606	2.000	0

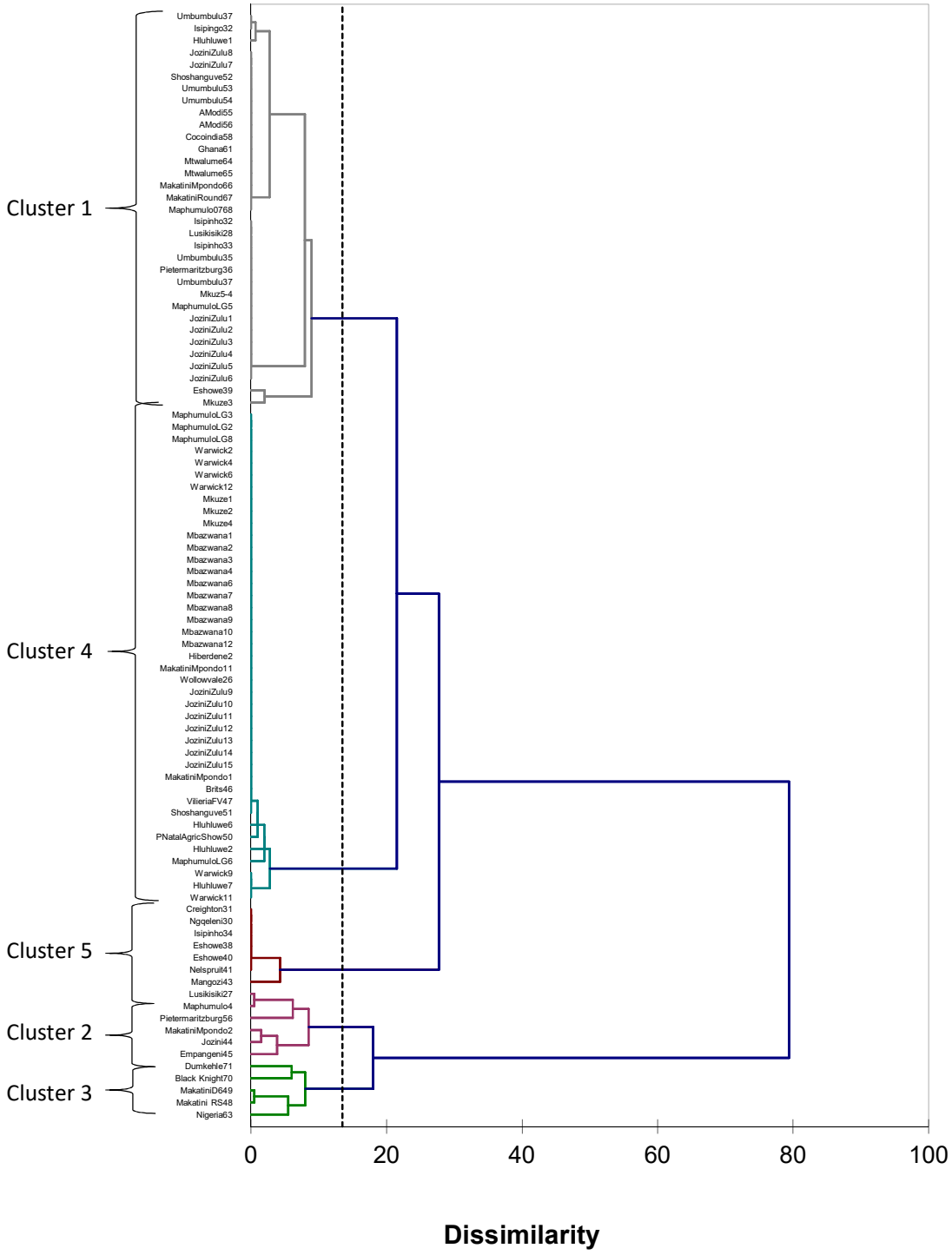

Figure 2.4: Agglomerative hierarchical clustering (AHC) of 86 South African taro accessions based on polymorphic SSRs. The Euclidean coefficient of dissimilarity between accessions are indicated on the X-axis. The accessions cluster in five clusters.

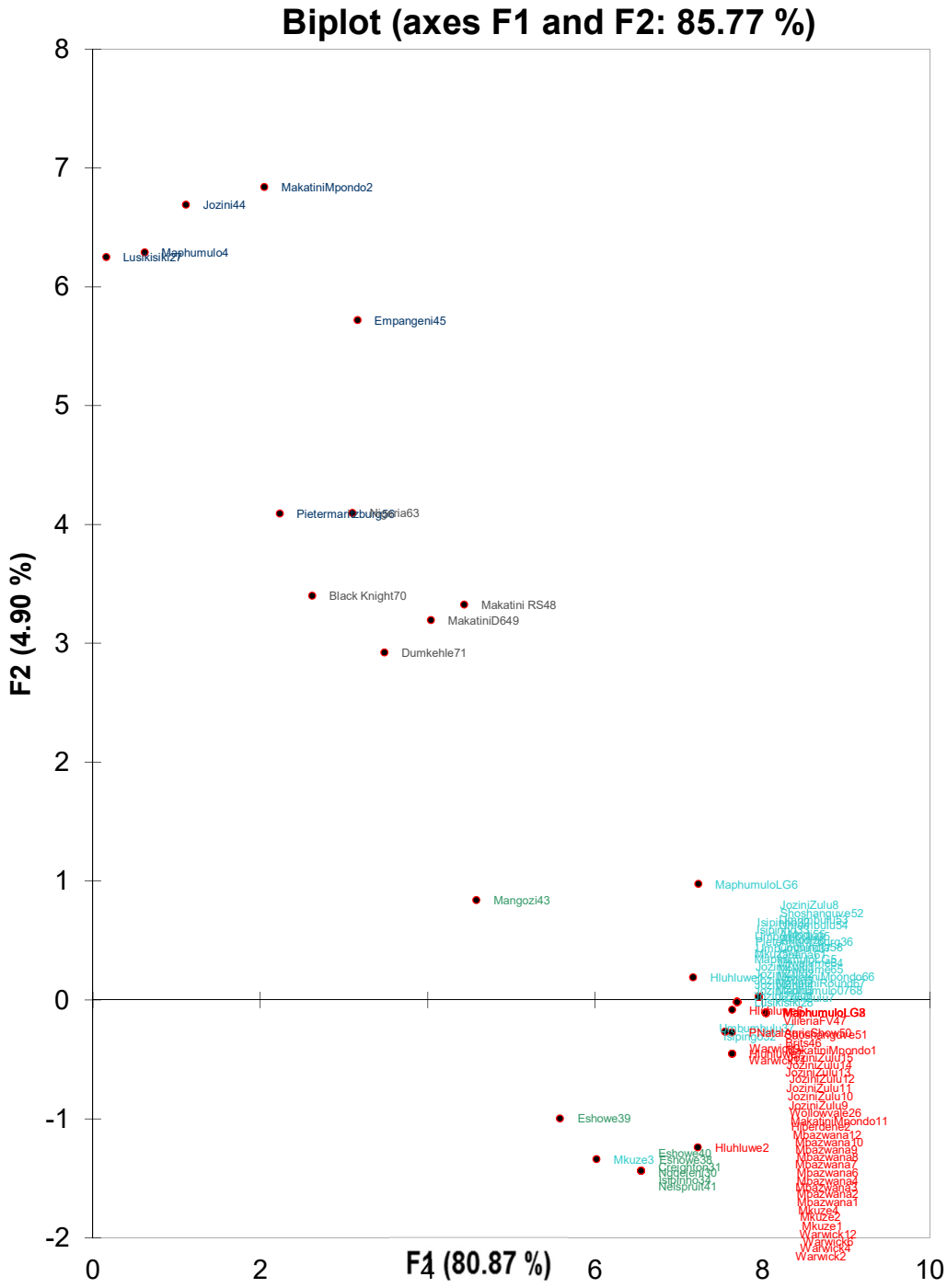

Figure 2.5: Biplot analysis of the polymorphic SSR loci. Cluster 1 from the cladogram is represented in cyan (cluster1), cluster 2 is represented in dark blue (cluster 2), cluster 3 is represented olive green (cluster3), cluster 4 is represented in red (cluster 4) and cluster 5 in teal (cluster 5). Cluster colours do not correspond to cluster colours in Figure 2.4.

Cluster 4 consist of 41 accessions with MaphumuloLG 2 the centre accession. All the accessions in this cluster are closely related, but MaphumuloLG 8, Warwick 2, 4, 6 and 12, Mkuze 1, 2 and 4, Mbazwana 1, 2, 3, 4, 6, 7, 8, 9, 10 and 12, Hibberdene 2, MakatiniMpondo 11, Willowvale 26, JoziniZulu 9, 10, 11, 12, 13, 14 and 15, MakatiniMpondo 1, Brits 46, VilieriaFV 47, and Shoshanguve 51 are apparently identical. This clustering included accessions collected in three different provinces of South Africa.

Accessions collected in Eshowe, Hluhluwe, Isipingo, Jozini, Lusiksiki, Makatini, Maphumolo, Mkuze and Pietermaritzburg were grouped in different clusters. All the accessions collected from Professor A Modi (University of KwaZulu-Natal), Mbazwana, Mtwalume, Umbumbulu and Warwick grouped in the same cluster. The ornamental accession, Black Knight, was placed in cluster 1 with other “edible accessions”. The accessions from Nigeria grouped in two different clusters within the South African accessions. All the accessions in cluster 3 are dasheen type.

The different accessions cluster together mostly according to the cladogram in a principal component biplot (Figure 2.5.) The first principal component of the biplot (Figure 2.5) explains the majority of the variation (80.87%). The second principal component only describes an additional 4.9% of the variation.

2.3 Discussion

A higher level of genetic diversity in the South African germplasm was observed when molecular analysis was performed than with morphological characterisation. No clear pattern was observed in the clustering of accessions in the cladogram. No correlation was detected between the different clusters and geographic distribution, since accessions from the same locality did not always cluster together, while conversely, accessions collected at different sites were grouped sometimes together. For example,

15 accessions were collected in Jozini (JoziniZulu type 1 to 15), and eight of these grouped in Cluster 1 and the others in Cluster 4. Cluster 1 and 4 are closely related according to the distances between the central accessions (Table 2). This absence of correlation with geography was also reported by Ivancic and Lebot (1999) in New Caledonia, Hartati et al. (2001) in Indonesia, Jianchu et al. (2001) in China, Matsuda and Nawata (2002) in eastern Asia, Hue et al. (2003), Kreike et al. (2004) in southeast Asia and the Pacific and Caillon et al. (2006) in Vanuatu.

Accessions that were almost identical on a molecular level were distinguished morphologically. For example, on a molecular level, Cocoindia 58, AModi 55 and 56 and Ghana 61 were identical; however, morphologically Cocoindia 58 is a more robust plant growing to twice the size of the other three accessions. The primers used do not offer definitive resolution of molecular differences between the genotypes. This indicated that in order to obtain more complete characterisation, both molecular and morphological data should be used. The cladograms clearly indicate that taro germplasm was exchanged extensively between different areas. Discussion with various farmers during fieldwork confirmed this, indicating that they obtained their planting material from other provinces and that extensive exchange of material take place. Some farmers even indicate getting planting material from Swaziland. There are also many accessions identified within the clusters that most probably are duplications. These accessions should be evaluated critically and to rationalise the number of accessions in the collection.

Although the results indicated that there is more diversity present in the local germplasm than expected when looking superciliously at the South African germplasm, the genetic base is still rather narrow when considering the amount of duplicates indicted in the analyses. This is also reported for other African countries such as Malawi (Mwenye et al. 2016), Ghana and Burkina Faso (Chaïr et al. 2016). Several authors reported that the

genetic diversity for taro seems to be low in Africa (Safo Kantaka 2004; Lebot 2009; Paul et al. 2011; Orji and Ogbona 2015; Chaïr et al. 2016).

2.4 Conclusion

South African taro still have a very narrow genetic base in spite of its long history South Africa. Farmers have selected landraces adapted to local conditions over time, but no diversity were introduced, except from neighbouring countries. The study shows that although the genetic base is narrow, more diversity exists than expected. The accessions were grouped in clusters, unfortunately no correlation exists between the clusters resulting from the morphological characteristics and molecular characteristics. Superior genotypes within each cluster can be used as parents and these superior landraces can also multiplied and distributed to farmers. Possible duplicates are also identified and the results can be used to rationalise the germplasm collection. In order to implement a successful breeding programme, it might be necessary widen the genetic base by importing germplasm. However, imported germplasm must be adapted to the local climatic conditions and acceptable for the local climatic consumers. One characteristic that showed low diversity in the South African germplasm is corm flesh colour. Yellow and purple fleshed taro, that have higher levels of beta-carotene and flavonoids respectively, can be imported for local evaluation.

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Chapter 3: Genetic improvement of *Colocasia esculenta* in South Africa

Abstract

Taro (*Colocasia esculenta*), known colloquially as Amadumbe in South Africa, is a tuber crop that is usually cultivated in the coastal and sub-tropical regions of South Africa. Taro is typically produced by smallholder farmers and traded informally, No improved cultivars exist in South Africa and farmers plant local landraces from material that has been retained from the previous season or traded and exchanged from other regions. The study aim to investigate to develop improved taro cultivars utilizing South African germplasm.

Fourteen taro genotypes were planted in a pan and fan glasshouse to be utilized as parents. GA_3 was used to artificially induce flowering. Eighty-five male female combination crossed were carried out. No offspring were obtained. This must be due to the triploid nature of the South African germplasm. It might be useful to pollinate diploid female parents with triploid male parents. The triploid male parents might produce balanced gametes at low percentages, which can fertilize the diploid female parents.

3.1 Introduction

There are three approaches to obtain improved taro cultivars (Sivan and Liyanage 1993). The easiest is to collect and evaluate local germplasm in order to identify promising lines to propagated and distributed. Alternatively, elite cultivars can be imported from other countries to evaluate under local conditions for suitability to local conditions and markets. Lastly, controlled breeding can be used to recombine characteristics in progeny that are evaluated against a set of predetermined criteria (Sivan and Liyanage 1993).

Most domesticated taro genotypes do not flower naturally (Del Peno 1990; Wilson 1990; Lebot 2009). Wild types do flower more easily and the character can be bred into a

population (Lebot 2009). The discovery of methods to induce flowering in taro has greatly facilitated breeding (Safo Kantaka 2004). One of the first breeding programmes was initiated in the early 1970's in the Solomon Islands to breed for taro leaf blight resistance (Patel *et al*. 1984, as cited by Lebot 2009). This was followed by breeding programmes in Hawaii, Samoa, Papua New Guinea (PNG), India, Philippines, Fiji and Vanuatu (Lebot 2009). There are taro breeding programmes in Mauritius that used mutation breeding to identify taro blight resistance (Seetohul et al. 2007). Lebot (2009) also noted that little was achieved in these programmes due to the narrow genetic base of the breeding stock and the introduction of "wild" germplasm that also introduced undesirable traits.

The aim of most taro breeding programmes is yield (Sivan and Liyanage 1993; Soulard et al. 2016), quality (Sivan and Liyanage 1993; Iramu et al., 2009) and pest and disease resistance (Sivan and Liyanage 1993; Iramu et al., 2009). Many taro breeders emphasis yield in the early generations of taro breeding programmes according to Soulard et al. (2016). According to Sivan and Liyanage (1993) it will take six to ten years to release a taro cultivar using traditional breeding methods. Modern biotechnology and molecular breeding tools can speed up the breeding. Recently, emphasis has also been placed on the nutrient composition of taro corms. Breeding is done to increase the nutrient content (bio-fortification), or decrease the anti -nutrient content of taro. These compounds are beta-carotene, anthocyanin antioxidants, phenolic compounds and oxalates etc. (Guchhait 2008; Champagne et al. 2013).

No improved taro cultivars exist in South Africa and farmers use traditional landraces. These landraces have been selected and maintained by local farmers over many years. Very little extra diversity is available to the farmers. Mare (2006) reported that there might only be four landraces present in KwaZulu-Natal. The objective of this study is to investigate the possibility to do hand pollinations, using South African taro landraces.

3.2 Materials and Methods

Ten local lines (Table 3.1) were planted in 50 cm pots at ARC-Roodeplaat in a pan and fan glasshouse. Ten plants of each of the fourteen plants were placed at random in a pan and fan glasshouse. The pots were watered daily by filling the pot to the rim. The lines were treated with 500 ppm gibberellic acid (Valent Biosciences). The gibberellic acid was sprayed on the leaves and petioles of the young plants when the plants had two mature leaves as described by Wilson (1990).

Table 3.1: Taro lines planted as parents (male or female) for cross hybridization.

Accession	Province/State	District	Nearest Town/village
2-2	KwaZulu-Natal	Hluhluwe	Hluhluwe
4	KwaZulu-Natal	Maphumulo	Maphumulo
5-2	KwaZulu-Natal	Mkuze	Mkuze
6-8	KwaZulu-Natal	Maphumulo	Maphumulo
7-5	KwaZulu-Natal	Durban Metro	Durban
9-11	KwaZulu-Natal	Jozini	Jozini
10-3	KwaZulu-Natal	Mbazwana	Mbazwana
26	Eastern Province	Mbhashe	Willowvale
28	Eastern Province	Ingquza	Lusikisiki
40	KwaZulu-Natal	Eshowe	Eshowe
44	KwaZulu-Natal	Umhlubuyalingana	Jozini
50	KwaZulu-Natal	Pietermaritzburg	Pietermaritzburg
56	KwaZulu-Natal	Pietermaritzburg	Pietermaritzburg
66	KwaZulu-Natal	Jozini	Jozini

Hand pollinations was done as described by Wilson (1990) and Lebot (2009). Hand-pollinations was done early in the mornings before 11:00. Inflorescences, to be used as female, were emasculated by catting away the spadix with a sharp knife. The emasculated inflorescence were covered with a muslin bag to prevent pollination by any other pollen sources. The male section of the inflorescence were then removed and discarded. Pollen were collected by removing the male part from inflorescence that have already shed pollen. Pollen was then transfer to the female flowers using a small brush

Table 3.2: Hand pollination done at ARC to produce taro seed.

Female	Pollen donor	Number of pollination
2-2	4	2
	5-2	3
	6-8	1
	26	3
	40	2
4	2-2	1
	6-8	3
	50	2
	66	4
5-2	2-2	2
	4	3
	7-5	2
	6-8	1
	26	2
	28	1
	40	
6-8	2-2	2
	66	2
7-5	2-2	2
	9-11	1
	44	3
	50	2
	66	2
9-11	44	2
	66	2
10-3	9-11	2
	44	2
	56	1
26	5-2	2
	40	1
	44	3
28	2-2	2
	5-2	2
	9-11	1
	40	1
	50	2
40	9-11	1
	50	2
	56	1
44	2.2	2
	50	2
50	9-11	2
	26	3
	28	2
56	9-11	2
	44	2
66	2-2	2
	5-2	1
	9-11	2
	40	1
	44	1

and the “female” inflorescence were covered again. The number of pollinations are summarised in Table 3.2.

3.3 Results and Discussion

The only natural flowerings at ARC-Roodeplaat were observed in two dasheen lines. However, the eddoe type is preferred for consumption in South Africa. All lines that were treated with gibberellic acid flowered.

Figure 3.1: Floral tissue in leaves of taro plants observed in plants four weeks after treatment with 500ppm gibberellic acid on line 26 (Photos: WS Jansen van Rensburg).

Figure 3.2: Flag leaves, the first indication of flowering. The plant on the left (line 26) was treated with gibberellic acid and the plant on right was a natural flowering clone from Vanuatu (Photos: WS Jansen van Rensburg).

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The first sign of the flowering was floral tissue that appeared in the leaves (Figure 3.1). This varied from small patches of yellow flower tissue, to whole leaves that were deformed and consisted of mostly floral tissue. The first flag leaves were visible four weeks after the plants were treated with gibberellic acid (Figure 3.2). The first flowers appeared two to four weeks later. The inflorescences appeared in clusters numbering between three to ten (Figure 3.3). The inflorescences of one cluster did not all open at the same time (Figure 3.3).

Figure 3.3: The cluster of inflorescences opening in sequence in Cocoindia. The first youngest inflorescence is closest to the petiole (Photos: WS Jansen van Rensburg).

All the inflorescences that developed were normal, and the male and female flowers were easily distinguished (Figure 3.4. 3.5 and 3.6). Various numbers of sterile female flowers were observed on an inflorescence. Flowering was therefore successfully induced as describe by Wilson (1990), Lebot (2009), Amadi et al. (2015) and Mukherjee et al. (2016). Some berries started to develop but aborted very soon due to no seed set (Figure 3.7). All hand pollinations were unsuccessful. Lebot (2009) and Mukherjee et al.

(2016) noted that the environment like dry winds, high temperatures and droughts, have a negative effect on pollination in taro. However, the mother plants were irrigated daily but the humidity in the glasshouse was low. Discussion with Dr A Ivancic (University of Maribor) and Dr V Lebot (CIRAD) indicated that the humidity might have been too low for successful hand pollination. All the South African germplasm tested by CIRAD in a collaborative project proved to be triploid (Chaïr et al. 2016). Triploids tend to form unbalanced gametes that can lead to low seed set.

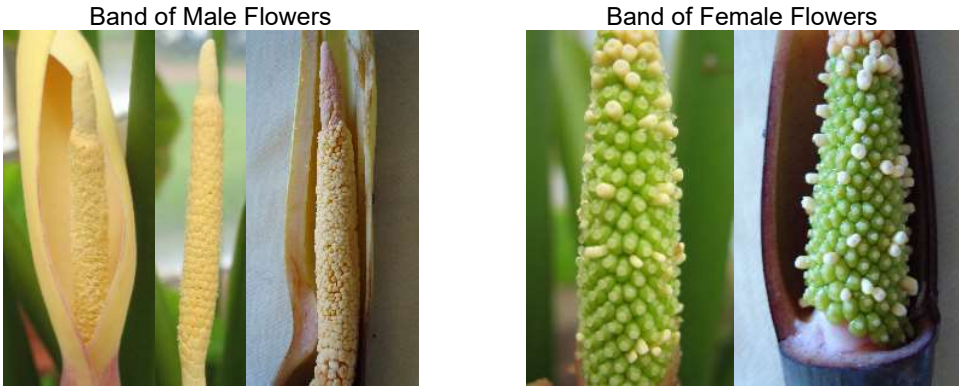

Figure 3.4: Detail of the inflorescence. Bands of male flowers in various stages can be seen on the left. The female flowers can be seen on the right. White to cream sterile flowers can be seen distributed between the green fertile flowers in the bands of female flowers (Photos: WS Jansen van Rensburg).

Figure 3.5 Cross section of the inflorescence. The sterile appendage can be seen at the far left, followed by the male flowers, a band of sterile flowers and a band of female flowers. Then the female flowers can be seen on the right. The fertile flowers are green and the infertile flowers, or staminates, are white (Photos: WS Jansen van Rensburg).

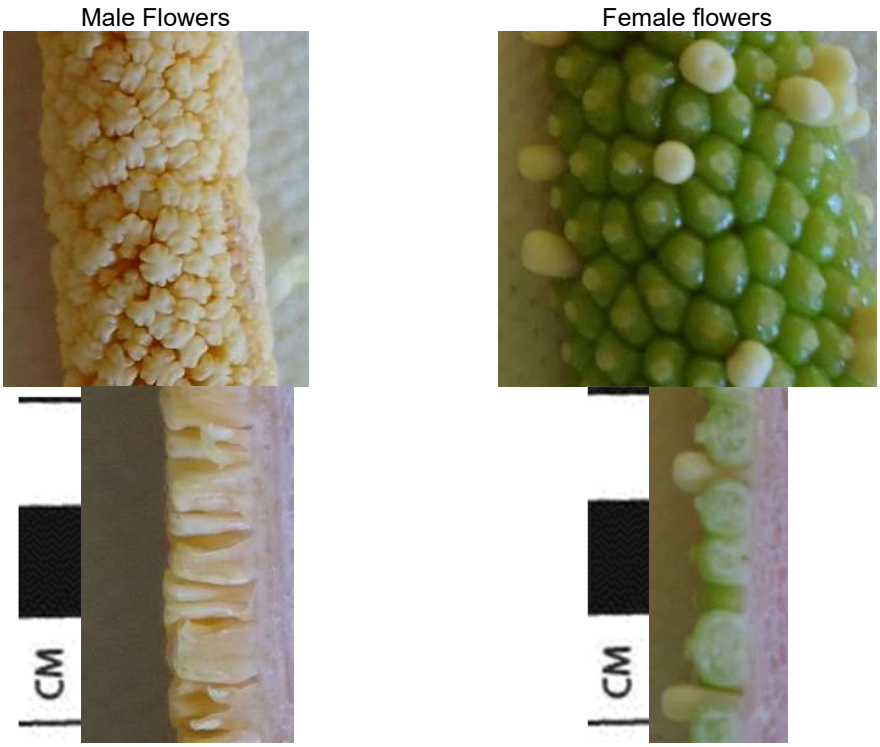

Figure 3.6: A close up of the male flowers (left) and female flowers (right). The green fertile female flowers can clearly be distinguished from the white sterile flowers on the lower part of the inflorescence (Photos: WS Jansen van Rensburg)

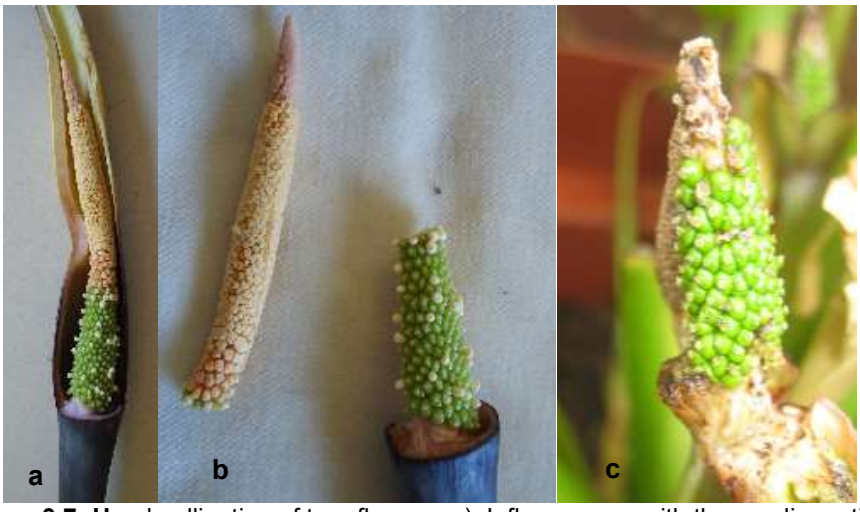

Figure 3.7: Hand pollination of taro flowers. a). Inflorescence with the spadix partially removed to show the position of the different parts of the inflorescence in relation to the spadix. b. Emasculation of the inflorescence. The upper band of male flowers completely cut off. c. The berries that start to develop after pollination. Unfertilised berries will abort soon after this stage. (Photos: WS Jansen van Rensburg).

3.4 Conclusion

Flowering was successfully induced into South African landraces using established technique that is used with success in all aroids (Mukherjee et al. 2016). However, no offspring were produced. This may be due to unfavourable climatic conditions or to the triploid nature of the germplasm. It is expected that triploids will have a very low percentage of balanced gametes, making breeding with them very difficult. In future breeding attempt, diploids will be used as the female parent with a South African triploid as the male parents. Crossed between diploids should be included to confirm the success of the pollination methodology. The assumption is that the possibility to successfully create a balanced gamete will be higher because thousands of pollen grains were formed during androgenises. A further study on the reproductive biology of the specific taro accessions in the ARC germplasm collection will also help to identify possible incompatibility mechanisms that prevent successful hybridization.

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Chapter 4: Genotype x Environment Interaction for *Colocasia esculenta* in South Africa

Abstract

Taro (*Colocasia esculenta*), commonly known as Amadumbe in South Africa, is a starch root crop that is traditionally cultivated in the coastal and sub-tropical regions of South Africa. Smallholder farmers are the main taro producers. Taro is generally traded in the informal market, however, taro has commercial potential. No improved cultivars exist and farmers plant local landraces of material that they have retain from the previous season. Furthermore, there is also very little information available on the influence of the environment on specific genotypes of taro. The aim of this study is to investigate the influence of different environments on selected taro landraces.

The Agricultural Research Council (ARC) has built up a taro germplasm collection that comprises of local and foreign accessions. Twenty-nine of these accessions were planted at three localities, representing different agro-ecological zones. These localities were Umbumbulu (South of Durban), Owen Sithole College of Agricultural (OSCA, Empangeni) and ARC - Vegetable and Ornamental Plants (Roodeplaat, Pretoria). Different growth and yield related parameters were measured. The data were analysed using analysis of variance (ANOVA) and additive main effects and multiplicative interaction (AMMI) analyses.

Significant GxE observed were between locality and specific lines for mean leaf length, leaf width, leaf number, plant height, number of suckers per plant, number of cormels harvested per plant, total weight of the cormels harvested per plant and corm length. No significant interaction between the genotype and the environment was observed for canopy diameter and corm breadth. From the AMMI model, it was clear that all the interactions were significant for leaf length, leaf width, number of leaves on a single plant,

plant height, number of suckers, number of cormels harvested from a single plant, weight of cormels harvested from a single plant. The AMMI model indicated that the main effects were significant but not the interactions with canopy diameter. The AMMI model for the length and width of the corms showed that effect of environment was highly significant but not effect of genotype.

There is a strong positive correlation between the number of suckers and the number of leaves (0.908), number of cormels (0.809) and canopy diameter (0.863) as well as between the number of leaves and the canopy diameter (0.939) and between leaf width and plant height (0.816).

There is not a single genotype that can be identified as “the best” genotype. This is due to the interaction between the environments and the genotypes. Amzam174 and Thandizwe43 seem to be genotypes that are often regarded as being in the top four. For the farmer, the total weight of the cormels harvested from a plant will be the most important. Thandizwe43, Mabhida and Amzam174 seem to be some of the better genotypes for the total weight and number of cormels harvested from a single plant. The local accessions also perform better than the foreign accessions. It is clear that some of the accessions do have the potential to be commercialised in South Africa based on wide adaptability and good corm yield.

4.1 Introduction

Fox et al. (1997) defined genotype by environment interaction (GxE) as the differential expression of a genotype across environments. Genotype refers to the expression of all the genes and interaction of those genes controlling a “character” or “trait”. Environment refers to the “external conditions” under which the plants grow. The environment consists of a combination of many external biological, physical, and time factors which vary independently or these external environmental factors can also interact with each other.

All these different external factors have an effect on the expression of the genotype to result in the specific phenotype observed (Romagosa and Fox 1993; Fox et al. 1997). GxE implies that the same genotypes may express their genetic potential in different ways under different environmental conditions. Various methods are used to evaluate GxE interactions. These methods vary from analysis of variance, regression to non-parametric methods like pattern analysis and multivariate techniques (Ramagosa and Fox 1993).

Two popular methods are the analysis of variance (ANOVA) and additive main effects and multiplicative interaction (AMMI). AMMIs was used to investigate GxE in taro (Eze et al. 201) and various other root and tuber crops like cassava (Dixon et al. 2002; Sholihin 2017), elephant foot jam (Kumar et al. 2014), potato (Steyn et al. 1993; Abalo et al. 2003; Hassanpanah 2009; Gedif and Yigzaw 2014), sweet potato (Calisckan et al. 2007; Kathabwalika et al. 2013) and yam (Egesi and Asiedu 2002; Otoo et al. 2006). Calisckan et al. (2007) is of the opinion that AMMI is better for evaluation than joint regression to evaluate GXE in sweet potato. Steyn et al (1993) noted that the AMMI graphical presentation of the results, to display the stability of a genotype in different environments, make interpretation of the data very easy. Egesi and Asiedu (2002) was able to select superior yam selections with specific or broad adaptation with the help of AMMI analysis.

Very little research has been published on GxE in taro. Eze et al. (2016) investigated the yield stability of eight taro genotypes across two locations in two years using AMMI and Genotype and Genotype-by-Environment (GGE) biplot models. Sing et al. (2006) used ANOVA to identify superior genotypes in a multi-location trials with six elite taro lines from the third cycle of the Papua New breeding program. ReyesCastro et al. (2005) investigated the performance of three purple cocoyam genotypes in four locations over two years.

Okpul (2005), Sing et al, (2006) and Eze et al. (2016) noted that taro corm yield was significantly affected by different environments, genotypes and by the interaction between the genotype by environments. Sing et al (2006) note that the environment influence TLB resistance. Lu et al. (2008) noted that environment has a significant influence on starch quality in taro. Eze et al. (2016) noted that not a single genotype can be identified as the best performer overall, but Sing et al. (2006) was able to identify a genotype that is superior over environments.

Some types of taro are adapted to paddy conditions, others to upland conditions, while some even tolerate relatively long periods of drought. Some are adapted to coastal areas or higher altitudes only (Lebot 2009). However, there is a lack of information on the influence of the environment on the expression of the genotypes. The aim of this study was to investigate the influence of three different agro-ecological environments on selected taro genotypes. The aim of the study is to establish the influence of three different environments on selected taro genotypes.

4.2 Materials and Methods:

4.2.1 Planting material

Whole corms of 29 different accessions were selected from the ARC germplasm collection. These accessions are described in Table 4.1. The genotypes include local and selected accessions of foreign germplasm.

4.2.2 Experimental layout

Four multi-locational trials were planted in areas representing diverse agro-ecological zones. These localities were Umbumbulu (South of Durban), Owen Sithole Agricultural College (OSCA, Empangeni), Lowveld College of Agriculture (Nelspruit) and ARC - Vegetable and Ornamental Plant Institute at Roodeplaat (Pretoria). The trial at

Umbumbulu was planted at a farmers field, while the other trials were planted at research institutions (Figure 4.1).

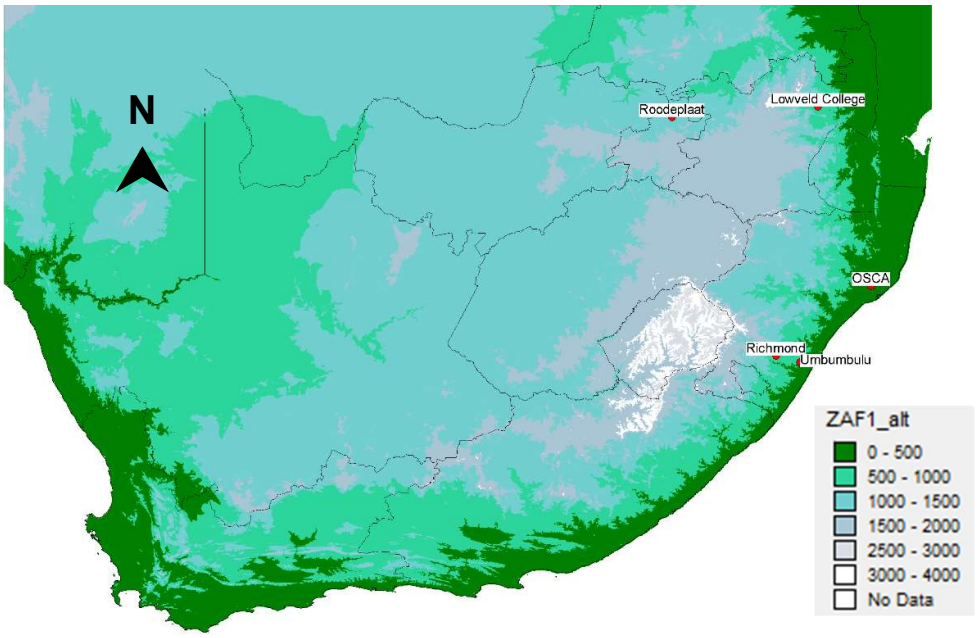

Figure 4.1: The distribution of the four trial sites. See text for local climate data. Map drawn with DIVA (Hijmans, Guarino, and Mathur, 2012).

Roodeplaat is situated outside Pretoria in Gauteng at an altitude of 1249 m above sea level. The coordinates are S25 36.914' E28 21.218'. The average annual minimum temperature is 10.1°C and the average annual maximum temperature is 25.5°C. The average annual rainfall is 691 mm for the period 1950 to 2000 (Hijmans at al. 2012).Roodeplaat has clay loam soils. Roodeplaat is situated within the warm temprate, winter dry, warm summers (Cwb) Köppen-Geiger climate classification zone (Conradie 2012).

Umbumbulu is situated south of Durban in KwaZulu-Natal at an altitude of 562 m above sea level. The coordinates are S30 00'50.3" E30 39'01.2". Over the period 1950 to 2000, the average annual minimum temperature was 13.3°C and the average annual maximum temperature 23.7°C, the average annual rainfall was 955 mm (Hijmans et al.

2012). The Umbumbulu farmers has loamy soils. OSCA is situated within the warm temprate, fully humid, warm summers (Cfb) Köppen-Geiger climate classification zone (Conradie 2012).

Table 4.1: Passport data on the collection sites of the genotypes included in the trials (N/A indicates data that are not available).

Line	Pro-vince	District	Nearest Town/village	Lati-tude	Longi-tude	Altit-ude
Maphumulo4	KZN[1]	Maphumulo	Maphumulo	-29.01	31.066	592
Maphumulo68	KZN	Maphumulo	Maphumulo	-29.01	31.066	592
Warwick72	KZN	Durban Metro	Durban	-29.85	31	10
DlomoDlomo171	KZN	Mthonjaneni	Empangeni	-28.72	31.88	359
Dlomodlomo173	KZN	Mthonjaneni	Empangeni	-28.72	31.88	359
Amzam174	KZN	Mthonjaneni	Empangeni	-28.72	31.88	359
Amzam182	KZN	Mthonjaneni	Empangeni	-28.72	31.88	359
Dlomodlomo19	KZN	Eshowe	Eshowe	-28.89	31.512	
Thandizwe43	KZN	Umhlab'uyalingana	Jozini	-26.95	32.75	30
Nkangala44	KZN	Umhlab'uyalingana	Jozini	-27.41	32.166	692
DlomoDlomo45	KZN	Mthonjaneni	Empangeni	-28.72	31.88	359
Vilieria47	KZN	Tshwane	Tshwane	-25.71	28.216	1283
Modi2	KZN	Pietermaritzburg	Pietermaritzburg	-29.61	30.4	662
Klang	Malaysia	N/A	N/A	N/A	N/A	
Ocha	KZN	N/A	N/A	N/A	N/A	N/A
Mhlongo	KZN	N/A	N/A	N/A	N/A	N/A
BongiweMkhize	KZN	N/A	N/A	N/A	N/A	N/A
BusisiweMkhize	KZN	N/A	N/A	N/A	N/A	N/A
Mabhida	KZN	N/A	N/A	N/A	N/A	N/A
Bhengu	KZN	N/A	N/A	N/A	N/A	N/A
Msomi	KZN	N/A	N/A	N/A	N/A	N/A
LungelephiMkhize	KZN	N/A	N/A	N/A	N/A	N/A
Gumede	KZN	N/A	N/A	N/A	N/A	N/A
Nxele	KZN	N/A	N/A	N/A	N/A	N/A
Ngubane	KZN	N/A	N/A	N/A	N/A	N/A
Mbili	KZN	N/A	N/A	N/A	N/A	N/A
Nkangala15	KZN	Umhlab'uyalingana	Jozini	-27.41	32.166	692
Nkangala16	KZN	Umhlab'uyalingana	Jozini	-27.41	32.166	692
DlomoDlomo14	KZN	Mthonjaneni	Empangeni	-28.72	31.88	359

[1] – KwaZulu-Natal

OSCA is situated in northern KwaZulu-Natal, northwest of Empangeni. OSCA is situated 65 m above sea level and the coordinates are S28 38'28.0" E31 55'47.3". During the 50

year period from 1950 to 2000, the average annual minimum temperature was 16.6°C and the average annual maximum temperature was 26.9°C. The average annual rainfall was 1030 mm. (Hijmans et al. 2012). OSCA has clay loam soils. OSCA is situated within the warm temprate, fully humid, hot summers (Cfa) Köppen-Geiger climate classification zone (Conradie 2012).

The Lowveld College of Agriculture is situated in the Northern outskirts of Nelspruit in Mpumalanga. It is situated 718 m above sea level. The coordinates are S 25 26'00.9" E30 58'26.2". The average annual minimum temperature was 13.5°C and the average annual maximum temperature was 26.2°C. The average annual rainfall was 793mm (Hijmans et al. 2012). The trial site is selected close to a little river and has clay soils. Frost does occur occasionally. Lowveld College of Agriculture is situated within the warm temprate, winter dry, hot summers (Cwa) Köppen-Geiger climate classification zone (Conradie 2012).

The soil at all sites was prepared by mechanical ploughing followed by ridging. Ridging was done mechanically with a "ridge maker", except at Umbumbulu where it was done by hand. The ridges were one meter apart and about 30 cm deep. Corms were planted between the ridges and then covered.

The plants were planted in a randomised complete block design with three replicates. Each plot consisted of three rows with five plants each (15 plants per plot). There was 1 m between the row spacing and 60 cm between the intra-row spacing. One cup well decomposed (150 ml) of compost was added before planting, and no additional fertilizer was added to the trials during the growth period, thus following the traditional planting method. All trials were irrigated three times a week for about 2 to 3 hours, to supply 15 mm of water per irrigation, trials were not irrigated when it was raining. The Umbumbulu

trail was rain fed. Weeding was done manually. The trial at the Lowveld Agricultural College was poorly maintained and was discarded from the analysis due to too many missing plants.

4.2.3 Data collection and Data analysis

Date for the characterization were not taken for the whole ARC taro germplasm collection, but only for the 30 accessions included in the multi-location trial. The following measurements were taken from three plants in each plot after four months of growth (Sing et al. 2008):

Emergence: All plants that did not emerged were counted. This parameter were mostly used when yield were calculated.

Leaf length: The length of the first unfolded mature leaf was measured with a tape measure. Measure was taken from the tip of the leaf, to the deepest point of the sinus.

Leaf width: width of the first unfolded mature leaf was measured with a tape measure across the widest part of the leaf.

Number of suckers: The number of suckers around the main plant were counted. In eddoe types, where it was difficult to recognise the main plant, the number of suckers were counted and then one was deducted from the total number. The number of suckers will correlate to the number of cormels.

Leaf number: All the mature leaves that were present on the main plant and suckers at that specific time were counted,

Plant height: The height of the tallest leaf of the plant was measured from soil level,

Canopy diameter (Canopy cover): The diameter of the whole plant, main plant with all suckers, was taken across the middle of the clump at the widest point.

Number of cormels: The total number of corms (dasheen type) or cormels (eddoe type) that were harvested was counted.

Weight of cormels: The total weight of all the corms (dasheen type) or cormels (eddoe type) harvested from one plant was determined.

Corm length: The average length of ten randomly selected corms (dasheen type) or cormels (eddoe type).

Corm breadth: The average breadth of ten randomly selected corms (dasheen type) or cormels (eddoe type) measured at its widest point.

A combined ANOVA was done for each of the characteristics across the localities using Genstat 14. The ANOVA gave an indication of significant differences between genotypes for the characteristics as well as GxE interactions. Further multivariate analysis (AMMI) was done where significant GxE was detected. The AMMIs gave an indication of the stability of the varieties for the different characteristics (Zobel et al., 1988). The AMMI analysis provides a biplot of main effects and the first principle component scores of the interactions (IPCA 1) of both genotypes and environments. The IPCA 1 score is on the vertical axis and the mean yield on the horizontal. Genotypes or environments that appear almost on a perpendicular line of the graph have similar means, while those that fall almost on a horizontal line have similar interaction patterns (Crossa, 1990). High PCA scores (either negative or positive as it is a relative value) indicate the specific adaptation of a genotype to certain environments, alternatively, the more the IPCA score approximates zero, the more stable the genotype is over all respective environments (Crossa, 1990).

4.3 Results

All data sets were complete (261 observations) except for “Leaf length” and “Number of suckers” where one outlier each was removed and two outliers were removed in “Corm length”. The ANOVA tables that were directly linked to yield parameters are presented in this chapter. All the other ANOVA tables are presented in Appendix 2. The AMMI, ANOVA tables and biplots that are directly linked to yield parameters are presented in this chapter. All the other AMMI ANOVA tables are presented in Appendix 4 and AMMI biplots are presented in Appendix 5.

4.3.1 Leaf length

The ANOVA table for leaf length is presented in Appendix 2. Significant interaction was observed between locality and specific lines for mean leaf length. On average, the leaves in Umbumbulu were significantly shorter than the leaves in Roodeplaat and OSCA. There were significant differences in mean leaf length between the different lines. The mean leaf length varied from 34.388 cm for BongiweMkhize to 24.916 for Klang.

The ANOVA for the AMMI (Appendix 4) model for leaf length also showed that the main effects and interactions were significant. IPCA1 explains 90.15% of the variation and IPCA2 9.855%. IPCA1 was significant, but IPCA2 was not significant (f=0.9993). The first four AMMI selections at OSCA were Mbili, BongiweMkhize, DlomoDlomo171, and Nkangala16, respectively. The first four lines at Roodeplaat were Thandizwe43, Ngubane, BongiweMkhize, and Gumede. The first four lines at Umbumbulu were Thandizwe43, Gumede, DlomoDlomo45, and Ngubane. No lines were in the top four lines in all three localities, but Thandizwe43, Ngubane, BongiweMkhize, and Gumede were in the top four in two localities. Overall the top four lines were BongiweMkhize, Mbili, Ngubane, and Thandizwe43. Thandizwe43 showed better stability than the other three. Gumede, Nkangala44, Bhengu, Mabhida, DlomoDlomo45, and Thandizwe43 showed good stability and above average leaf length while Mhlongo, Modi2, Ocha, Dlomodlomo173, Msomi and Amzam182 showed good stability but below average leaf length. Maphumulo68 and Klang showed low stability and below average leaf length, while Mbili also has low stability but above average leaf length (Appendix 5).

4.3.2 Leaf width

The ANOVA table for leaf width is presented in Appendix 3. The influence of genotype, location, and the interaction between genotype and environment was significant. There were significant differences between OSCA and the other two localities for mean leaf

width. Leaves were significantly wider in OSCA. The overall mean leaf width was 25.49cm. The mean leaf width varied from 34.00cm measured for Mbili in OSCA, to 15.77cm for Klang in OSCA (Data not shown). Mean leaf width also differed significantly between lines. Ngubane has the widest leaves (28.79cm) and Klang has the narrowest leaves (20.37 cm).

From the ANOVA table for the AMMI model, it is clear that all the main effects and interactions are significant (Appendix 4). IPCA1 explains 78.25% of the variation and IPCA2 21.75%. IPCA1 is significant, but IPCA2 is not significant (f=0.9065). It is therefore meaningful to plot the AMMI1 biplot. The first four AMMI selections at Umbumbulu and Roodeplaat were Mabhida, Ngubane, Gumede, and Bhengu. The first four lines selected at OSCA were Mbili, Amzam174, Vilieria47, and Ngubane. Overall Ngubane, Mbili, Mabhida and Amzam174 had the highest mean leaf width; however, Mabhida and Amzam174 were less stable. Gumede, Nkangala15 Ngubane, and Mabhida had good stability and above average leaf width. Nkangala44. Mhlongo, Dlomodlomo173, Msomi, and DlomoDlomo45 showed good stability but below average leaf width (Appendix 5).

4.3.3 Leaf number

The ANOVA for leaf number is presented in Appendix 3. The influence of genotype, location and the interaction between genotype and environment was significant. All three localities differed significantly for leaf number. The plants in OSCA had, on average, more leaves than at Umbumbulu, but less leaves than at Roodeplaat. The mean number of leaves per plant was 26.51. The highest mean number of leaves was 57.33 observed for Amzam174 at Roodeplaat, while the lowest mean number of leaves was 6.67 for DlomoDlomo1713 observed in Umbumbulu. The average number of leaves also differed between the different lines. Amzam174 has the highest average number of leaves (34.297) and Nxele the lowest average number of leaves (20.149).

From the ANOVA table (Appendix 5) for the AMMI model for the number of leaves on a single plant, it is clear that the main effects and the interactions were significant. IPCA1 explains 63.56% of the variation and IPCA2 36.44%. IPCA1 was significant, but IPCA2 was not significant (f=0.3493). Therefore, only AMMI1 plots were drawn. According to the AMMI, the four best lines at OSCA were Thandizwe43, BusisiweMkhize, LungelephiMkhize, and Ngubane. The four best lines at Roodeplaat were Amzam174, Thandizwe43, Nkangala15, and Amzam182. The four best lines at Umbumbulu were Amzam174, Amzam182, Bhengu, and Nkangala15. No lines were in the top four at all three localities but Amzam174, Thandizwe43, and Nkangala15 were under the top four in two of the three localities, namely Roodeplaat and Umbumbulu. The overall four best lines were Amzam174, Thandizwe43, Nkangala15, and Dlomodlomo19. Amzam174 showed a high degree of stability, while Thandizwe43 showed low stability. Bhengu, Amzam182, Amzam174, and Nkangala15 showed a high degree of stability and above average number of leaves, while Vilieria47 and DlomoDlomo171 also showed a high degree of stability but below average number of leaves. LungelephiMkhize, BusisiweMkhize, and Thandizwe43 showed a low degree of stability but above average number of leaves, while Maphumulo4 showed low degree of stability and below average number of leaves per plant (Appendix 5).

4.3.4 Plant height

The ANOVA table for plant height is presented in Appendix 3. The influence of locality and genotype was highly significant, the interaction between the locality and the genotype was significant but not as highly as the two factors individually (p=0.0547). The height of the plants differed significantly between all three localities. The plant heights differ significantly and the tallest plants were observed in OSCA, followed by Roodeplaat, and then Unbumbulu. The overall mean for plant height was 73.55cm. The plant height varied between103.44cm for BongiweMkhize in OSCA, to 51.33cm for Maphumulo68 in

OSCA. On average, the tallest plants were observed for Ngubane (85.63cm) and shortest plants were Maphumulo68 (59.45cm).

The ANOVA table (Appendix 4) for AMMI model for plant height showed that the main effects and interactions were significant. IPCA1 explains 79.22% of the variation and IPCA2 20.78%. Only IPCA1 is significant and not IPCA2 (f=0.9397). AMM2 plots will therefore not be meaningful. According to the AMMI, the four best lines in OSCA were BongiweMkhize, Ngubane, Nkangala16, and Amzam174. The four best lines in Roodeplaat were Amzam174, Ngubane, Thandizwe43, and Nkangala15 and the four best lines in Umbumbulu were Amzam174, Thandizwe43, Ngubane and DlomoDlomo14. Amzam174 and Ngubane were in the top four lines in all three localities while Thandizwe43 was in the top four lines in two localities. Ngubane, Amzam174, Thandizwe43, and BongiweMkhize had the best overall performance for plant height. However, BongiweMkhize and Ngubane showed high levels of instability. The most stable lines for plant height were Amzam182, Modi2, Mhlongo, and Dlomodlomo173, but only Amzam182 showed above average plant height. The highest levels of instability were observed in Maphumulo4, DlomoDlomo14, Maphumulo68, and BongiweMkhize. BongiweMkhize was ranked fourth overall for plant height but was also the fourth least stable line (Appendix 5).

4.3.5 Canopy diameter

The ANOVA table for canopy diameter is presented in Appendix 3. The influence of the genotypes and environment was significant, but not the interaction between the environment and the genotype (p=0.566) (Appendix 3).There were significant differences in mean canopy diameter between the localities (Appendix 3).There were also significant differences in mean canopy diameter between the lines (Appendix 3). Thandizwe43 has the highest mean canopy diameter (86.630cm) and Maphumulo68 the lowest canopy diameter (61.811cm). The overall mean canopy diameter was 74.98cm.

The canopy diameter varied between 113.89cm for Amzam182 at Roodeplaat, and 36.58cm for Maphumulo682 at OSCA.

The ANOVA table for the AMMI model indicated that the main affects were significant. However, the interactions were not significant (f=0.4964). IPCA1 explains 62.92% of the variation and IPCA2 37.08%. Neither IPCA1 (f=0.2289) or IPCA2 (f=0.7916) were significant. The AMMI model for canopy diameter was therefore not important. An AMMI biplot were drawn even though the model is not significant. According to the AMMI, the four best lines in OSCA were Amzam174, Thandizwe43, Dlomodlomo19, and BongiweMkhize in descending order. At Roodeplaat it was Thandizwe43, Nkangala44, Amzam174, and Amzam182 in descending order, and in Umbumbulu it included Nkangala44, Thandizwe43, Bhengu, and Amzam182. Thandizwe43 was in the four best lines in all three localities, while Nkangala44, Amzam174, and Amzam182 were in the top four in two localities. Overall, Thandizwe43, Amzam174, and Nkangala44 were the top lines. Nkangala44 had the lowest performance but was more stable than the other two. Maphumulo68 had the poorest performance and showed the least stability. Amzam182, Mbili, and Mabhida showed good stability and above average yield.

4.3.6 Number of suckers

The ANOVA table for the number of suckers per plant is presented in Appendix 3. The influence of environment, genotype and the interaction between environment and genotype was significant. There were significant differences in the mean number of suckers between the different localities, Roodeplaat had the highest mean number of suckers per plant, followed by OSCA, and then Umbumbulu. There were also significant differences between the lines for the mean number of suckers per plant. Amzam174 had the highest number of suckers (14.56) and Maphumulo68 the lowest (6.78). The overall mean number of suckers per plant was 10.27. The mean number of suckers per plant varied from 25.33 at Roodeplaat for Mbili, to 2.11 at Umbumbulu for Warwick72.

According to the ANOVA table (Appendix 4) for the AMMI model, for the number of suckers per plant, the main effects were highly significant and the interaction was also significant. IPCA1 explains 55.36% of the variation and IPCA2 44.64%. IPCA1 was significant but IPCA2 was not (f=0.1279).

According to the AMMI, the four first lines at Umbumbulu were Amzam174, Nkangala44, Amzam182, and Thandizwe43. The best four lines at and Roodeplaat were Amzam174, Nkangala15, DlomoDlomo171, and Thandizwe43. Amzam174 and Thandizwe43 ranked first and fourth respectively at all three localities. Overall, the four best lines were Amzam174, Nkangala15, Thandizwe43, and DlomoDlomo171. Amzam182 and Nkangala16 showed a very high degree of stability and above average number of suckers. BongiweMkhize, LungelephiMkhize, Gumede, and Warwick72 also showed a high degree of stability but below average number of suckers. DlomoDlomo171 and Nkangala15 showed a high degree of instability and above average number of suckers, while Maphumulo68 and Vilieria47 also showed high instability, but below average number of suckers.

4.3.7 Number of cormels harvested from a single plant

The ANOVA table for the number of cormels harvested per plant is presented in Table 4.4 and Appendix 3. The influence of environment, genotype and the interaction between environment and genotype was significant (Table 4.2). The mean number of cormels harvested per plant differed significantly between the localities. The highest mean number of corms per plant was harvested at Roodeplaat and lowest at Umbumbulu (Table 4.3). Significant interactions were observed between the mean number of cormels and locality (Table 4.4). The overall mean number of corms per plant was 22.17 corms. The highest mean number of cormels observed was 62.67 for DlomoDlomo171 in Roodeplaat and the lowest mean number of cormels were observed was 2.11 for Klang in OSCA.

Table 4.2: ANOVA table for the mean number of cormels of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	31001.855	15500.927	334.72	<.0001
Rep(Loc)	6	884.555	147.425	3.18	0.0055
Line	28	15354.256	548.366	11.84	<.0001
Loc*Line	56	10443.248	186.486	4.03	<.0001

Table 4.3: The t-grouping for mean number of cormels harvested per plant in the different localities. Means with the same letter were not significantly different. Critical value of t = 1.97419 and LSD = 2.037.

Loc	Mean	Std Dev	N	t Grouping
Roodeplaat	34.697	16.393	87	A
OSCA	23.691	11.039	87	B
Umbumbulu	8.131	3.1815	87	C

Table 4.4: The t-grouping for mean number of cormels for the different lines. Critical Value of t = 1.97419 and LSD = 6.3332. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping												
Dlomodlomo19	35.111	21.997	9	A												
DlomoDlomo171	34.557	24.906	9	A												
Amzam174	33.370	17.841	9	A	B											
Nkangala16	32.443	20.503	9	A	B	C										
Amzam182	31.019	21.727	9	A	B	C	D									
DlomoDlomo14	30.038	18.769	9	A	B	C	D	E								
Thandizwe43	29.297	15.915	9	A	B	C	D	E								
Ocha	28.149	16.226	9		B	C	D	E								
Nkangala15	27.814	17.014	9		B	C	D	E								
Nkangala44	26.853	15.212	9			C	D	E								
Modi2	26.482	13.861	9			C	D	E								
DlomoDlomo45	25.556	18.905	9				D	E	F							
Dlomodlomo173	25.222	18.634	9				D	E	F	G						
Maphumulo4	24.352	20.579	9					E	F	G	H					
Mabhida	19.853	9.504	9						F	G	H	I				
Nxele	19.147	15.728	9							G	H	I				
Vilieria47	18.183	9.046	9								H	I	J			
Gumede	17.557	8.042	9									I	J	K		
Mhlongo	17.370	9.156	9									I	J	K		
BongiweMkhize	17.148	12.075	9									I	J	K		
Bhengu	17.074	7.940	9									I	J	K		
BusisiweMkhize	16.851	8.668	9									I	J	K		
Ngubane	16.480	7.854	9									I	J	K	L	
Warwick72	16.073	11.158	9									I	J	K	L	
LungelephiMkhize	14.722	8.649	9									I	J	K	L	
Msomi	12.557	7.091	9										J	K	L	M
Mbili	11.667	5.479	9											K	L	M
Maphumulo68	10.167	4.626	9												L	M
Klang	7.907	4.915	9													M

The main effects and the interaction was highly significant according to the ANOVA for the AMMI model (Table 4.5 and Appendix 4). IPCA1 explains 79.78% of the variation and IPCA2 20.22%. Both IPCA1 and IPCA 2 were significant.

According to the AMMI, the four first lines at Roodeplaat were DlomoDlomo171, Dlomodlomo19, Nkangala16, and Amzam182. The first four lines at OSCA were Dlomodlomo19, Amzam174, DlomoDlomo171, and Nkangala16 and the first four lines at Umbumbulu were Amzam174, Dlomodlomo19, Thandizwe43, and Modi2. Dlomodlomo19 was under the top four lines at all three localities, while Amzam174 and Nkangala16 were under the top four lines in two of the three localities. Overall, Dlomodlomo19, DlomoDlomo171, Amzam174, and Nkangala16 were the first four lines for number of cormels harvested from a single plant. However, all four of these lines show low stability. Modi2, Thandizwe43, Nkangala15, Ocha, and Nkangala44 showed a high degree of stability and above average number of cormels harvested from one plant. BongiweMkhize, Nxele, and Mhlongo also showed a high degree of stability but below average number of cormels harvested from a single plant. DlomoDlomo171 and Amzam182 showed high degree of instability but above average number of cormels harvested from one plant, while Mbili and Klang also showed a high degree of instability but lower than average number of cormels harvested from a single plant (Figure 4.2 and Appendix 5).

Table 4.5: ANOVA table for AMMI model for the number of cormels harvested from the single plant.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	65464	252		
Treatments	86	56799	660	14.26	<0.001
Genotypes	28	15354	548	11.84	<0.001
Environments	2	31002	15501	105.14	<0.001
Block	6	885	147	3.18	0.0055
Interactions	56	10443	186	4.03	<0.001
IPCA 1	29	8332	287	6.20	<0.001
IPCA 2	27	2111	78	1.69	0.0248
Residuals	0	0			
Error	168	7780	46		

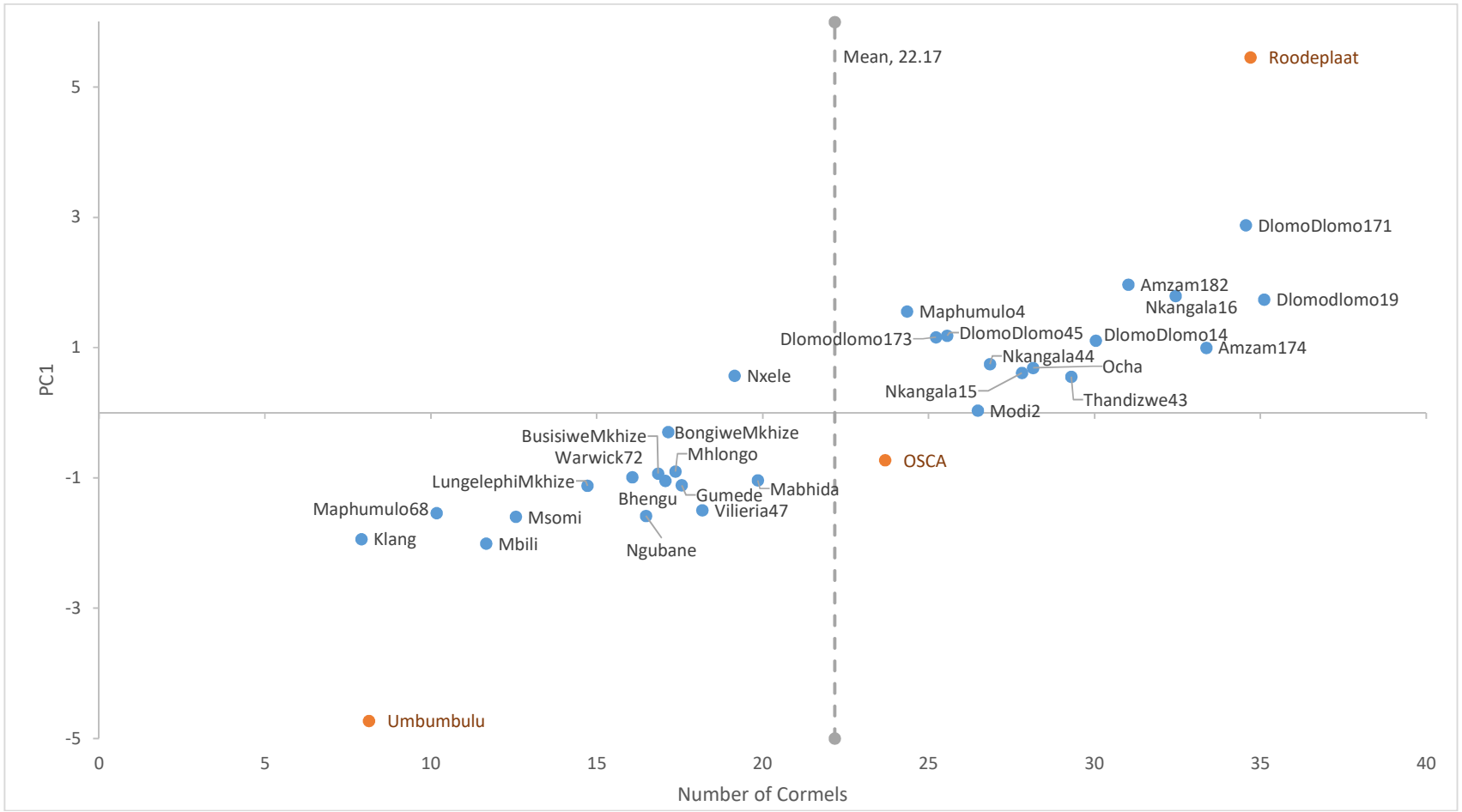

Figure 4.2: The AMMI1 model for number of cormels, plotting the overall mean of each line and locality against the first principal component (PC1).

4.3.8 Weight of cormels harvested from a single plant

The ANOVA table for the total weight of the cormels harvested per plant is presented in table 4.6 and Appendix 3. The influence of environment, genotype and the interaction between environment and genotype was significant (Table 4.6). The mean weight of cormels harvested per plant was significantly lower in Umbumbulu than in Roodeplaat and OSCA. There was no significant difference between Roodeplaat and OSCA (Table 4.7). The mean weight of cormels also differed significantly between lines (Table 4.8). The highest mean weight was for Thandizwe43 (0.90kg) and the lowest was Klang (0.3589). The overall mean weight of all the cormels harvested from one plant was 0.654kg. The mean weight of cormels harvest per plant varied from 1.44kg for Ngubane in OSCA to 0.09g for Klang in OSCA.

Table 4.6: ANOVA table for the mean weight of cormels harvested from a single plant of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	14.680	7.340	150.19	<.0001
Rep(Loc)	6	0.9764	0.162	3.33	0.0040
Line	28	3.3429	0.119	2.44	0.0003
Loc*Line	56	6.1053	0.109	2.23	<.0001
LocxLin	0	0.0000	.	.	.

Table 4.7: The t-grouping for mean weight of cormels harvested per plant in the different localities. Means with the same letter were not significantly different. Critical Value of t = 1.97419 and LSD = 0.0662

Loc	Mean	Std Dev	N	t Grouping
Roodeplaat	0.84747	0.243	87	A
OSCA	0.79644	0.381	87	A
Umbumbulu	0.32080	0.108	87	B

The ANOVA table (Table 4.9 and Appendix 4) for AMMI model for the weight of the cormels harvested from a single plant showed that the main effects and the interactions were significant. IPCA1 explains 82.27% of the variation and IPCA2 17.73%. IPCA1 was highly significant while IPCA2 was not significant (f=0.7209).

From the AMMI analysis, the four top lines at Roodeplaat were Amzam174, Mhlongo, Maphumulo4, and Thandizwe43. The four top lines at Umbumbulu were Thandizwe43, Mabhida, Amzam174, and Gumede. The four top lines at OSCA were Ngubane, Thandizwe43, Mabhida, and Vilieria47. Thandizwe43 was under the top four lines in all three localities while Mabhida, and Amzam174 were under the top four lines in two localities. Thandizwe43, Mabhida, Amzam174, Ngubane were overall the top four lines. Ngubane showed a very high degree of instability while Amzam174 showed a high degree of stability. Nxele, Dlomodlomo19, Gumede, and BusisiweMkhize were the most stable lines but Nxele had a below average weight for cormels harvested from a single plant (yield). Ngubane, Klang, Vilieria47, and Mbili were the least stable lines.

Table 4.8: The t-grouping for mean weight of cormels harvested per plant for the different lines. Critical Value of t = 1.97419 and LSD = 0.2057. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping						
Thandizwe43	0.9000	0.466	9	A						
Mabhida	0.8656	0.401	9	A	B					
Amzam174	0.7867	0.343	9	A	B	C				
Ngubane	0.7833	0.512	9	A	B	C				
Gumede	0.7689	0.359	9	A	B	C	D			
BusisiweMkhize	0.7478	0.494	9	A	B	C	D	E		
Mhlongo	0.7333	0.387	9	A	B	C	D	E		
Bhengu	0.7322	0.290	9	A	B	C	D	E		
Dlomodlomo19	0.6944	0.316	9	A	B	C	D	E		
Modi2	0.6878	0.335	9		B	C	D	E		
LungelephiMkhize	0.6856	0.388	9		B	C	D	E		
Ocha	0.6833	0.322	9		B	C	D	E		
DlomoDlomo14	0.6622	0.361	9		B	C	D	E		
Nkangala15	0.6533	0.364	9			C	D	E		
BongiweMkhize	0.6478	0.402	9			C	D	E		
Amzam182	0.6422	0.370	9			C	D	E		
Nkangala16	0.6256	0.283	9			C	D	E		
DlomoDlomo45	0.6200	0.348	9			C	D	E		
Nxele	0.6144	0.337	9			C	D	E		
Vilieria47	0.6111	0.368	9			C	D	E		
Mbili	0.6044	0.330	9			C	D	E	F	
Warwick72	0.5944	0.399	9			C	D	E	F	
DlomoDlomo171	0.5922	0.287	9			C	D	E	F	
Nkangala44	0.5844	0.200	9			C	D	E	F	
Maphumulo4	0.5833	0.367	9			C	D	E	F	
Msomi	0.5756	0.324	9				D	E	F	
Dlomodlomo173	0.5522	0.406	9					E	F	G
Maphumulo68	0.4011	0.217	9						F	G
Klang	0.3589	0.244	9							G

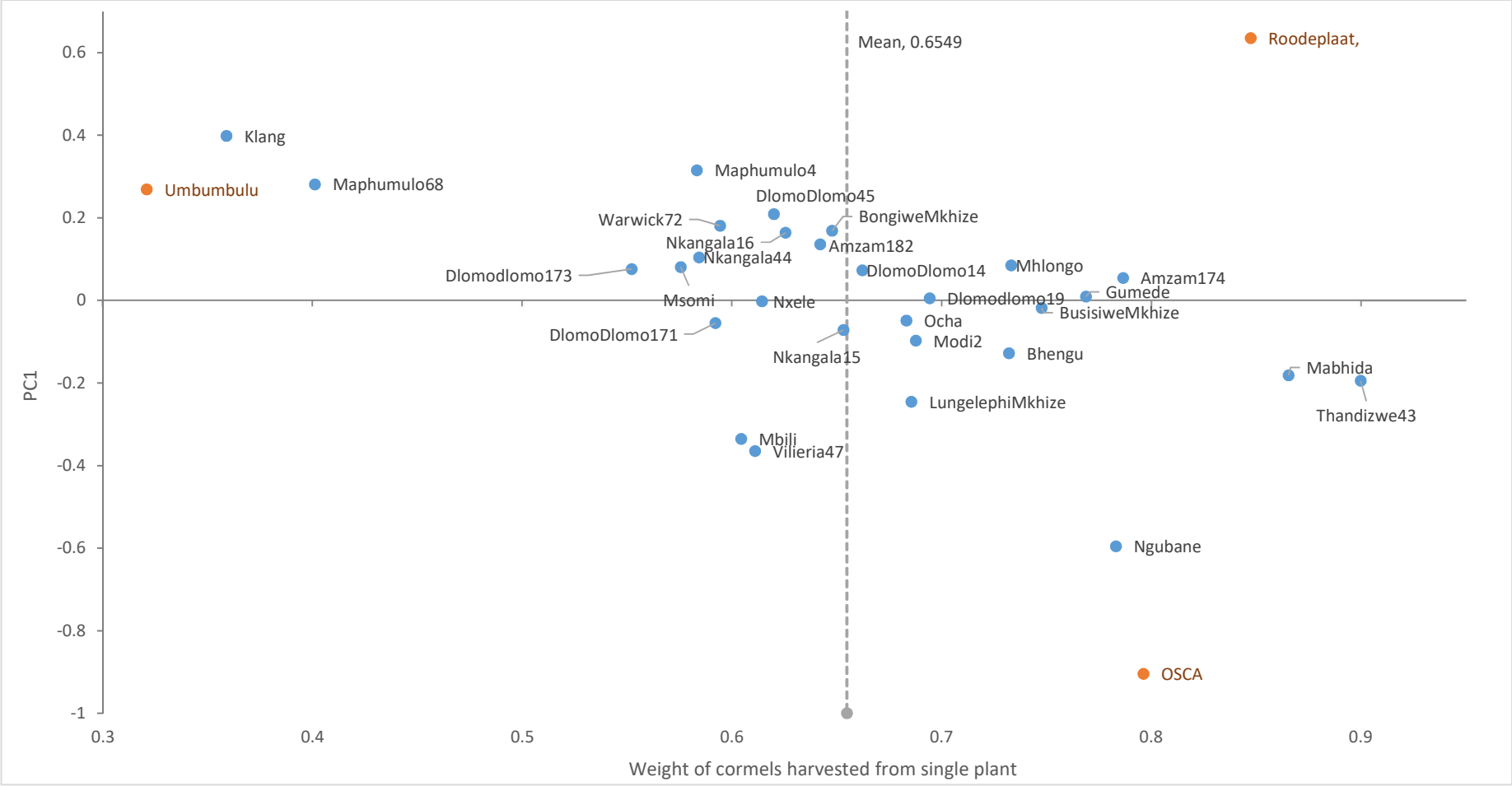

Figure 4.3: The AMMI1 model for weight of cormels harvested from a single plant, plotting the overall mean of each line and locality against the first principal component (PC1).

Table 4.9: ANOVA table for AMMI model for the weight of the cormels harvested from a single plant.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	33.315	0.1281		
Treatments	86	24.128	0.2806	5.74	<0.001
Genotypes	28	3.343	0.1194	2.44	<0.001
Environments	2	14.680	7.3400	45.10	<0.001
Block	6	0.976	0.1627	3.33	0.0040
Interactions	56	6.105	0.1090	2.23	<0.001
IPCA 1	29	5.023	0.1732	3.54	<0.001
IPCA 2	27	1.083	0.0401	0.82	0.7209
Residuals	0	0.000			
Error	168	8.211	0.0489		

All these lines, except Ngubane, showed below average weight for cormels harvested from a single plant. Ngubane showed the highest degree of instability but was ranked overall fourth for weight of cormels harvested from a single plant (Figure 4.3 and Appendix 5).

4.3.9 Corm length

The ANOVA table for corm length is presented in Appendix 3. The effect of locality was highly significant but the effect of genotype (p=0.1885) was not, however, the interaction between the genotype and the environment was significant (p=0.0368). The mean corm length differed significantly between the different localities. There were also some differences between lines for the mean corm length. The overall mean corm length was 67.63mm. The mean corm length varied between 103.20mm for LungelephiMkhize at OSCA, and 41.43mm for Nkangala443 at Roodeplaat.

The ANOVA table (Appendix 4) for AMMI model for the length of the corms showed that effect of environment was highly significant but not effect of genotype. IPCA1 explains 76.01% of the variation and IPCA2 23.99%. Only IPCA1 was significant while IPCA2 was not significant (f=0.8206). From the AMMI analysis, the four top lines at Umbumbulu were Maphumulo68, LungelephiMkhize, Ngubane, and Ocha in descending order. The four top lines at Roodeplaat were LungelephiMkhize, Maphumulo68, Ngubane, and Ocha. The same four lines were identified at Roodeplaat and Umbumbulu, but the

ranking was different. The top four lines at OSCA were Amzam174, Nkangala16, LungelephiMkhize, and Nxele. LungelephiMkhize was under the top four lines in all three localities while Maphumulo68, Ngubane and Ocha were under the top four in only two localities. Overall, the four best lines were LungelephiMkhize, Amzam174, Nxele, and Thandizwe43. All four lines showed more or less the same level of stability. Ocha, Bhengu, Ngubane, and Warwick72 showed good stability and above average corm length, while DlomoDlomo171, Modi2, Gumede, Dlomodlomo19, Maphumulo4, and Vilieria47 showed good stability but below average corm length. Mabhida, Amzam174, Maphumulo68, and Nkangala16 showed the lowest levels of stability for corm length over the three localities (Appendix 5).

4.3.10 Corm breadth

The ANOVA table for mean corm breadth is presented in Appendix 3. The influence of environment was significant on corm breadth but not the influence of genotype (0.0617) and the interaction between the environment and genotype (p=0.118). The mean breadth of the corms in Umbumbulu and Roodeplaat were significantly broader than in OSCA. The corm breadth also varied between the different genotypes. The overall mean breadth of a corm was 45.136mm. The mean corm breadth varied from 82.08cm observed in OSCA for Nkangala16, to 29.95cm observed in Roodeplaat for Nkangala44.

The ANOVA table (Appendix 4) for AMMI model for the breadth of the corms showed that the effect of the environment was highly significant, but not the effect of the genotypes. IPCA1 explain 81.49% of the variation and IPCA2 18.06%. IPCA1 was significant, but not IPCA2 (f=9866). According to the AMMI, the top four lines at Roodeplaat were Nxele, Klang, Gumede, and Amzam174, in descending order. The top four lines in Umbumbulu were Nxele, Gumede, Klang, and Amzam174, in descending order. The same four lines were top in Roodeplaat and Umbumbulu, but the ranking was different. The top four lines in OSCA were Nkangala16, LungelephiMkhize, Nxele, and

Amzam174, in descending order. Nxele and Amzam174 were under the top four lines in all three localities, while Klang and Gumede were under the top four lines in two localities. Overall the best genotypes were Nxele, Amzam174, Gumede, Nkangala16, and LungelephiMkhize, but Nxele and Gumede showed more stability. Ngubane, Bhengu, Warwick72, Dlomodlomo19, Gumede, and Thandizwe43 had good stability and above average corm breadths. Modi2, Maphumulo4, Mbili, Mabhida, and BusisiweMkhize had good stability but below average come breadths.

LungelephiMkhize, Msomi, Nkangala15 had above average corm breadth but showed low stability. Klang showed low stability and a mean corm breadth below average, although it was under the top four lines in Umbumbulu. Generally, lines were less stable for corm breadths than corm length.

4.3.11 Summery of the ANOVA and AMMI results

The summary of the four best genotypes for each characteristic as determined by the ANOVA (Table 4.10 and Appendix 6) and AMMI (Table 4.11 and Appendix 6) show that there is not a single genotype that can be identified as “the best”. This is due to the interaction between the environments and the genotypes. Eze et al. (2016) also noted that the ranking of Nigerian taro cultivars differ between the environments. Amzam174 and Thandizwe43 seem to be genotypes that are often under the top four. For the farmer, the total weight of the cormels harvested from a plant will be often the most important. Consumer preference may sometimes play an important role in a farmer’s choice of cultivar as well. Thandizwe43, Mabhida and Amzam174 seem to be some of the better genotypes. Table 4.15 combines the best cultivars as indicated by the ANOVA and the AMMI analysis. There is no clear line that can be identified as the best performer, however Amzam174, DlomoDlomo45 and 19 and Thandizwe43 occur several times for the yield related parameters. Egesi and Asiedu (2002) were also not able to identify one single yam genotype that perform best over all the environments.

Table 4.10: Summary of the four top genotypes in the three different localities and overall taken from the ANOVA analysis. Each cultivar is marked with a colour to make it easier to follow specific genotype.

Characteristic	Top genotype	Second best genotype	Third best genotype	Fourth best genotype
Leaf Length	BongiweMkhize	Mbili	Ngubane	Thandizwe43
Leaf Width	Ngubane	Mbili	Mabhida	Amzam174
Leaf number	Amzam174	Thandizwe43	Nkangala15	DlomoDlomo45
Plant Height	Ngubane	Amzam174	Thandizwe43	BongiweMkhize
Canopy diameter	Thandizwe43	Amzam174	Nkangala44	Amzam182
Number of suckers	Amzam174	Nkangala15	Thandizwe43	DlomoDlomo171
Number of cormels	DlomoDlomo45	DlomoDlomo171	Amzam174	Nkangala16
Weight of cormels	Thandizwe43	Mabhida	Amzam174	Ngubane
Corm length	LungelephiMkhize	Amzam174	Nxele	Ngubane
Corm breadth	Nxele	Amzam174	Gumede	Nkangala16

Table 4.11: Summary of the four top genotypes in the three different localities and overall taken from the AMMI analysis. Each cultivar is marked with a colour to make it easier to follow specific genotype. Rankings that are different than rankings in figure 4.12 is printed in red.

Characteristic	Best Performer	Second Best Performer	Third Best Performer	Fourth Best Performer
Leaf Length	BongiweMkhize	Mbili	Ngubane	Thandizwe43
Leaf Width	Ngubane	Mbili	Mabhida	Amzam174
Leaf Number	Amzam174	Thandizwe43	Nkangala15	Dlomodlomo19
Plant Height	Ngubane	Amzam174	Thandizwe43	BongiweMkhize
Canopy Diameter	Thandizwe43	Amzam174	Nkangala44	Amzam182
Number of suckers	Amzam174	Nkangala15	Thandizwe43	DlomoDlomo171
Number of Cormels	Dlomodlomo19	DlomoDlomo171	Amzam174	Nkangala16
Weight of Cormels	Thandizwe43	Mabhida	Amzam174	Ngubane
Corm Length	LungelephiMkhize	Amzam174	Nxele	Thandizwe43
Corm Breadth	Nxele	Amzam174	Gumede	Nkangala16

Table 4.12 summarizes the best performers in the three different localities as well as overall. The most stable and unstable cultivars are also indicated in Table 4.12. Ngubane can be recommended to plant in OSCA, based on weight of corms harvested, but it lacks stability, it may not perform as well in other areas. Thandizwe43 and Amzam174 can be recommended for planting at Roodeplaat and similar environments. Mabhida and

Table 4.12: Summary of the best genotypes according to the ANOVA and the AMMI analysis for each characteristic in each locality as well as the most stable and unstable genotype for each characteristic. Each cultivar is marked with a colour to make it easier to follow specific cultivars.

Characteris-tic	Locality	ANOVA Best Performer	AMMI Best Performer	Stable	Unstable
Leaf Length	OSCA	Mbili	Mbili		
	Roodeplaat	Thandizwe43	Thandizwe43		
	Umbumbulu	Vilieria47	Thandizwe43		
	Overall	BongiweMkhize	BongiweMkhize	Gumede	Klang
Leaf Width	OSCA	Mbili	Mbili		
	Roodeplaat	Gumede	Mabhida		
	Umbumbulu	Bhengu	Mabhida		
	Overall	Ngubane	Ngubane	Nkangala44	Klang
Leaf Number	OSCA	Thandizwe43	Thandizwe43		
	Roodeplaat	Amzam174	Amzam174		
	Umbumbulu	Amzam174	Amzam174		
	Overall	Amzam174	Amzam174	DlomoDlomo171	LungelephiMkhize
Plant Height	OSCA	BongiweMkhize	BongiweMkhize		
	Roodeplaat	Ocha	Amzam174		
	Umbumbulu	Amzam174	Amzam174		
	Overall	Ngubane	Ngubane	Amzam182	Maphumulo4
Canopy Diameter	OSCA	Amzam174	Amzam174		
	Roodeplaat	Amzam182	Thandizwe43		
	Umbumbulu	Nkangala44	Nkangala44		
	Overall	Thandizwe43	Thandizwe43	Mabhida	Maphumulo68
Number of Suckers	OSCA	Amzam174	Amzam174		
	Roodeplaat	Mbili	Amzam174		
	Umbumbulu	Amzam174	Amzam174		
	Overall	Amzam174	Amzam174	Amzam182	DlomoDlomo171
Number of Cormels	OSCA	Dlomodlomo19	Dlomodlomo19		
	Roodeplaat	DlomoDlomo171	DlomoDlomo171		
	Umbumbulu	Amzam174	Amzam174		
	Overall	DlomoDlomo45	Dlomodlomo19	Modi2	DlomoDlomo171
Weight of Cormels	OSCA	Ngubane	Ngubane		
	Roodeplaat	Thandizwe43	Amzam174		
	Umbumbulu	Mabhida	Thandizwe43		
	Overall	Thandizwe43	Thandizwe43	Nxele	Ngubane
Corm Length	OSCA	LungelephiMkhize	Amzam174		
	Roodeplaat	BusisiweMkhize	LungelephiMkhize		
	Umbumbulu	LungelephiMkhize	Maphumulo68		
	Overall	LungelephiMkhize	LungelephiMkhize	DlomoDlomo171	Nkangala16
Corm Breadth	OSCA	Nkangala16	Nkangala16		
	Roodeplaat	Nxele	Nxele		
	Umbumbulu	Klang	Nxele		
	Overall	Nxele	Nxele	Ngubane	Nkangala16

Thandizwe43 can be recommended for planting in Umbumbulu and similar environments. Nxele can be recommended for general planting in different areas as they are the most stable for yield (total weight of cormels) although it is not under the highest performer for yield.

4.3.12 Correlation between variables

There is a strong positive correlation between the number of suckers and the number of leaves (0.908), number of cormels (0.809) and canopy diameter (0.863) (Table 4.13). The positive correlation between the number of corms and the number of suckers is expected because each sucker has its own corm. There is also a very strong positive correlation between the number of leaves and the canopy diameter (0.939) and between leaf width and plant height (0.816) (Table 4.12). There is also a positive correlation between leaf length and leaf width (0.697), leaf length and plant height (0.746), number of suckers and plant height (0.643), leaf number and the number of cormels (0.739), number of cormels and canopy diameter (0.787) and corm length and corm breadth (0.784) (Table 4.12). In general, there is a positive correlation between all parameters that indicate photosynthesis area and parameters that increase yield.

Table 4.13: The correlation between the variables.

Variables	Leaf-Length	Leaf-width	No Suck-ers	Leaf no	Plant height	No Corm-els	Cano-py diam	Corm length	Corm breath	Weight cormels
Leaf length	**1.000**									
Leaf width	**0.697**	**1.000**								
No suckers	0.456	0.261	**1.000**							
Leaf no	0.445	0.187	**0.908**	**1.000**						
Planthght	**0.746**	**0.816**	**0.643**	0.574	**1.000**					
NoCormels	0.437	0.202	**0.809**	**0.739**	0.515	**1.000**				
Canopydiam	0.502	0.194	**0.863**	**0.939**	0.568	**0.787**	**1.000**			
Cormlgth	0.131	0.496	-0.176	-0.342	0.251	-0.234	-0.395	**1.000**		
Cormbrth	0.170	0.540	0.118	-0.063	0.462	0.014	-0.159	**0.784**	**1.000**	
Wtcormels	0.553	0.536	0.764	0.759	0.731	0.716	0.690	0.109	0.330	**1.000**

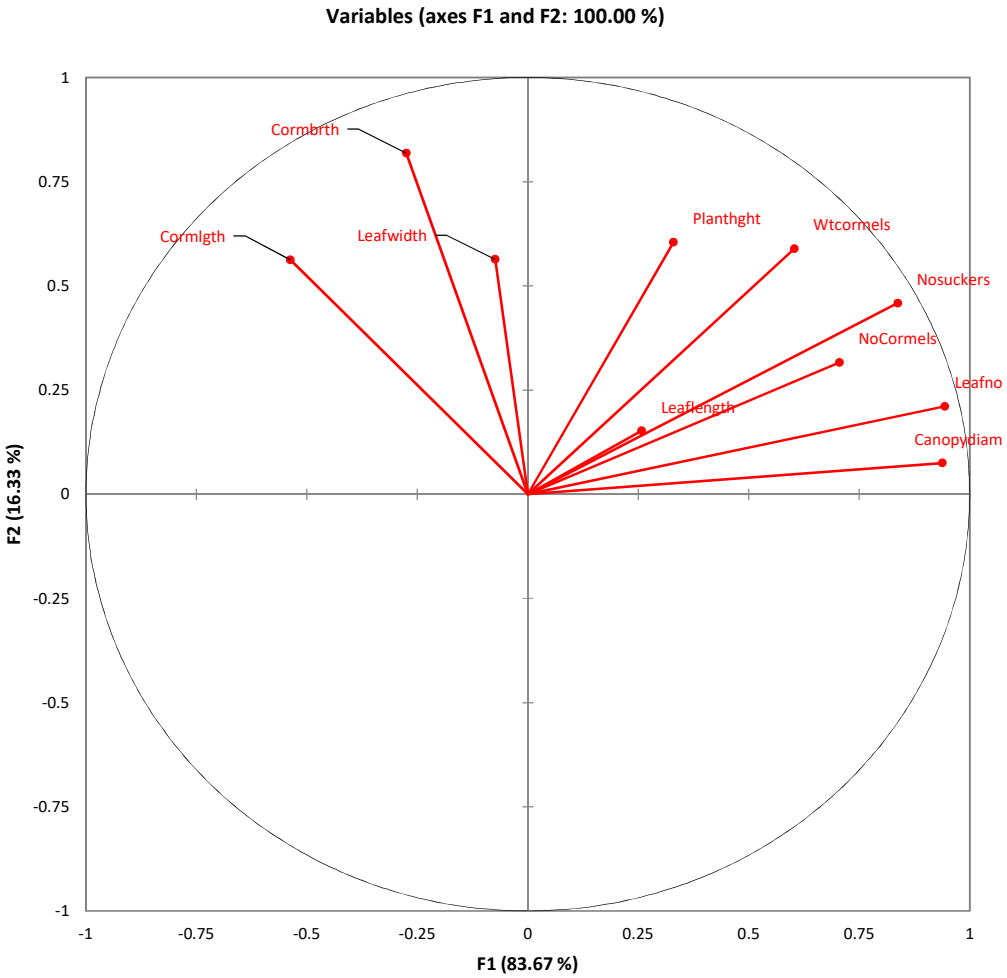

Figure 4.4: The biplot showing the correlation between the different characteristics.

There is a strong correlation on the biplot (Figure 4.4) between most of the characteristics. The number of cormels and the number of suckers are highly correlated. This is to be expected because each sucker is exists of a cormel and the leaves growing from the cormel. The weight of the cormels harvested is strongly correlated to the number of cormels. This give an indication that both these characteristics can be used to select for high yielding eddoe type genotypes. Canopy diameter, plant height and leaf number is to an extend correlated to characteristics related to yield like corm weight and number. This indicate that the area of available to photosynthesis can influence yield.

4.4 Discussion

The ANOVA showed that the environment had a significant influence on all the characteristics except for the length and breadth of the cormels. This might be because the cormels form continuously during the season (Lebot 2009) and therefore do not mature at the same time. Furthermore, the secondary and tertiary cormels were also harvested, the secondary and tertiary cormels vary in size and shape depending on when and there they were initiated. Cormels of various sizes can be found at the same time on a plant. The position where the cormel develop, influences the shape of the cormel as well. Cormels that form lower on the mother corm must grow around the cormels that have developed higher up on the mother corm. The influence of environment was highly significant for all characteristics. These cormels will be more elongated and curved. Significant interaction between environment and tuber yield were noted in other tuberous crops like cassava (Dixon et al. 2002; Sholihin 2017), elephant foot jam (Kumar et al. 2014), potato (Abalo et al. 2003; Hassanpanah 2009; Gedif and Yigzaw 2014), sweet potato (Caliskan et al. 2007; Kathabwalika et al. 2013) and yam (Egesi and R. Asiedu 2002; Otoo et al. 2006).

Ngubane can be recommended to plant in OSCA, based on weight of corms harvested, but it lacks stability, it may not perform as well in other areas. Thandizwe43 and Amzam174 can be recommended for planting at Roodeplaat and similar environments. Mabhida and Thandizwe43 can be recommended for planting in Umbumbulu and similar environments. Nxele can be recommended for general planting in different areas as they are the most stable for yield (total weight of cormels) although it is not under the highest performer for yield. Egesi and Asiedu (2002) and Kapinga et al. (2009) also identified superior yam selections with specific or broad adaptation using AMMI analysis. The ranking of the genotypes for specific trait was not consistent between environments. This was also noted by Eze et al. (2016) in taro, (Osiru et al. 2009) in sweet potato.

The interaction between the environment and genotype was also significant for all characteristics except the height of the plants, the canopy diameter, and corm breadth. The interaction was highly significant to the number of cormels per plant and the weight of the cormels harvested from a plant. These two characteristics are the major indicators of yield. It is an indication that although these characteristics can be improved by breeding, the environment must also be taken into account during evaluation. It will, therefore, be advantageous to select for taro genotypes in different environments.

All the characteristics are positively correlated. There is a strong positive correlation between the number of suckers and the number of leaves, number of cormels and canopy diameter. There is also a positive correlation between leaf length and leaf width, leaf length and plant height, number of suckers and plant height, leaf number and the number of cormels, number of cormels and canopy diameter and corm length and corm breadth. The positive correlation between the number of corms and the number of suckers is expected because each sucker has its own corm. The weight of the cormels harvested is strongly correlated to the number of cormels. This give an indication that both these characteristics can be used to select for high yielding eddoe type genotypes.

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Chapter 5: General Discussion

Amadumbe (*Colocasia esculenta*) is a popular starchy crop in certain parts of South Africa (Modi 2007). Amadumbe is better known as taro; dasheen; eddoe; cocoyam or elephant ear in other parts of the world (Safo Kantaka 2004). It is a popular starchy staple in tropical Africa, Asia, Pacific Islands and Americas (Lebot 2009). However, despite its wide distribution range, taro is still regarded as an orphan crop (Lebot 2009). In South Africa, taro is mostly cultivated in the subtropical eastern coastal and lowland areas. There are no commercial taro cultivars in South Africa and research on taro is inadequate when compared with that of conventional root and tuber crops (Modi, 2007). The present study has highlighted certain aspects of the diversity of taro present in South Africa and its implication on breeding. The study attempted to establish the genetic diversity of taro in South Africa, to generate diversity by means of hand pollinations and to determine the influence of the environment on taro landraces.

The diversity of South African taro landraces were determined by agro-morphological and SSR characterization. In the South African germplasm, a higher level of diversity was revealed by molecular studies than what was revealed by morphological characterisation. No clear clustering of accessions was observed in the cladogram for both morphological and molecular studies. No correlation was detected between the different clusters and geographic distribution; accessions from the same locality did not always cluster together, or conversely, accessions collected at different sites were grouped together. In other regions Ivancic and Lebot (1999), Hartati, et al. (2001), Jianchu et al. (2001), Matsuda and Nawata (2002), Hue et al. (2003), Kreike, van Eck and Lebot (2004) Caillon, et al. (2006) who detected little correlation between geographic distribution and diversity of taro, and little correlation between dendrograms based on molecular data and dendrograms based on morphological data. In some instances, accessions that were almost identical on a molecular level were distinguishable

morphologically. Hartati et al. (2001) also found no clear correlations on dendrograms based on morphological characteristics, isozymes and RAPDs, but Sing et al. (2011) did observe a correlation between results obtained with morphological traits, RAPDs and SSRs. Trimanto et al. (2010) also detected a significant correlation between isozyme data and morphological data. A narrow genetic base is also reported for other African countries such as Malawi (Mwenye et al. 2016), Ghana and Burkina Faso (Chaïr et al. 2016). Several authors reported that the genetic diversity for taro seems to be low in Africa (Safo Kantaka 2004; Lebot 2009; Paul et al. 2011; Orji and Ogbona 2015; Chaïr et al. 2016). Together, previous studies and the present results indicated that in order to obtain more complete characterisation, a range of molecular and morphological data should be considered. The present results clearly indicate that taro germplasm was exchanged extensively between different areas. Discussions with various farmers confirmed this as they indicated that they obtained their planting material from other provinces. Farmers in Mtwalume (KwaZulu-Natal) indicated that they have obtained planting material from relatives in Lusikisiki in the Eastern Cape. Farmers in Jozini indicated that they have obtained planting material from Umbumbulu and the Natal South Coast. However, there is no formal seed system and all these exchange is informal. The present results indicated that there is more diversity present in the local germplasm than expected, however, the genetic base is still rather narrow.

The study also attempt to create diversity within the South African taro germplasm. Flowering was induced successfully in the South African germplasm; however, no successful pollinations were obtained. The failure to get off spring might be due to unfavourable conditions during pollination or to the ploidy level of the South African landraces. Discussions with Dr A Ivancic (University of Maribor) and Dr V Lebot (CIRAD) indicate that the humidity might be too low for successful hand pollinations. All the South African germplasm tested by CIRAD in a collaborative project also proved to be triploid (Chaïr et al. 2016). It is expected that triploids will have a very low percentage of

balanced gametes, making breeding with them very difficult. In future breeding attempt, diploids will be used as the female parent with a South African triploid as the male parents, this might increase the success of the pollinations. Crosses between diploids should be included to confirm the success of the pollination methodology. A further study on the reproductive biology of the specific taro accessions in the ARC germplasm collection will also help to identify possible incompatibility mechanisms that prevent successful hybridization.

The present study also intended to determine the influence of environment on the taro landraces. The study showed clearly that environment had a significant influence on the morphological expression in the South African taro germplasm. The exception was the breath of the cormels. This might be because the cormels are forming continuously during the season and therefore do not mature at the same time. The position where the cormel develops influences the shape of the cormel as well. Cormels that form lower on the mother corm must grow around the cormels that have developed higher up on the mother corm.

The interaction between the environment and genotype was also significant for all characteristics except for plant height and the canopy diameter. The interaction was highly significant for characteristics related to yield such as the number of cormels per plant and the weight of the cormels harvested from a plant. This indicate that the environment should be taken into account when breeding for these characteristics.

No single genotype could be identified as “the best” by the ANOVAs or the AMMIs. This showed that the environment influence the expression of genotypes in taro. Amzam174 and Thandizwe43 seem to be two genotypes that are often in the top four for all 10 characteristics. In terms of the total weight of cormels harvested from a plant

Thandizwe43, Mabhida and Amzam174 seem to be some of the better genotypes. When the ANOVA and AMMI “best performing genotypes” were combined, no top performer could be identified. However, Amzam174, DlomoDlomo45 and 19 and Thandizwe43 occur several times for the yield related parameters. Ngubane can be recommended to plant in OSCA, but, because it lacks stability, it may not perform as well in other areas. Thandizwe43 and Amzam174 can be recommended for planting at Roodeplaat and similar environments. Mabhida and Thandizwe43 can be recommended for planting in Umbumbulu and similar environments. Nxele can be recommended for general planting in different areas as they are the most stable for yield (total weight of cormels) although it is not under the highest performer for yield. Nqubane is better adapted to the Owen Sitole College of Agriculture, in the northern KwaZulu-Natal environmental conditions. The only foreign cultivar in the study, Klang, has low yields in all environments. Klang had a much longer growing season than the local germplasm, and had not matured when the other germplasm were harvested. Furthermore, Klang is a dasheen type cultivar. The dasheen type is generally more popular warmer regions such as the Pacific Islands and Indonesia, however, South African consumers do prefer eddoe type.

All the characteristics morphological are positively correlated. There are a strong positive correlation between the number of suckers and the number of leaves, number of cormels and canopy diameter. There was also a positive correlation between leaf length and leaf width, leaf length and plant height, number of suckers and plant height, leaf number and the number of cormels, number of cormels and canopy diameter and corm length and corm breadth. The weight of the cormels harvested was strongly correlated to the number of cormels. This gave an indication that both these characteristics could be used to select for high-yielding eddoe type cultivars.

The results on the diversity studies indicate that there is diversity in the South African taro landraces that can be exploited. It was also clear that the environment influence the

expression of various traits. There was no landrace identified that prove to be the best performing over all environments, but there is high yielding landraces that proof to be stable over environments. These landraces can be multiplied for the use by farmers and can be included in future breeding attests. However, the inability to produce any offspring from the hand pollinations hamper the creation of new diversity through hand pollinations. Further investigation in the reproductive biology of taro in South Africa is warranted.

To implement a successful breeding programme it is necessary to import more diploid germplasm to widen the genetic base. However, choice of germplasm must be done with caution to ensure that the imported germplasm is adapted to South African climate and that it is acceptable for the South African consumers. Superior genotypes within each cluster in the dendrograms can be identified and used as parents in a clonal selection and breeding programme. If triploids within the South African germplasm are to be used in further breeding, a diploid must be used as the female parent and a triploid can used as the male parents. The assumption is that the change to get a balanced gamete is much larger among the thousands of pollen grains produced. It is therefore necessary to determine the ploidy of the South African germplasm. Further study on the reproductive biology of specific taro accessions in the ARC germplasm collection will also help to identify possible incompatibility mechanisms that prevent successful breeding.

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Appendix 1: ARC taro Germplasm collection (Not all of the accessions was included in the analysis).

ARC ID	Subspecies	ame	Collectors no	Country of collection	Province/State	District	Nearest Town/village	Latitude	I
2-1	Edoe	Hluhluwe	2-1	South Africa	KwaZulu-Natal	Hluhluwe	Hluhluwe	-28.016	
2-2	Edoe	Hluhluwe	2-2	South Africa	KwaZulu-Natal	Hluhluwe	Hluhluwe	-28.016	
2-6	Edoe	Hluhluwe	2-6	South Africa	KwaZulu-Natal	Hluhluwe	Hluhluwe	-28.016	
2-7	Edoe	Hluhluwe	2-7	South Africa	KwaZulu-Natal	Hluhluwe	Hluhluwe	-28.016	
3	Edoe	3083 E2	3	South Africa	KwaZulu-Natal	Durban Metro	Durban	-30.883	
4	Edoe	Maphumulo, Kwadeka	4	South Africa	KwaZulu-Natal	Maphumulo	Maphumulo	-29.015	
5-2	Edoe	Mkuze market #2	5-2	South Africa	KwaZulu-Natal	Mkuze	Mkuze	-27.6	
5-3	Edoe	Mkuze market #3	5-3	South Africa	KwaZulu-Natal	Mkuze	Mkuze	-27.6	
5-4	Edoe	Mkuze market #4	5-4	South Africa	KwaZulu-Natal	Mkuze	Mkuze	-27.6	
6-4	Edoe	Maphumulo (Lina Gayani)	6-4	South Africa	KwaZulu-Natal	Maphumulo	Maphumulo	-29.015	
6-5	Edoe	Maphumulo (Lina Gayani)	6-5	South Africa	KwaZulu-Natal	Maphumulo	Maphumulo	-29.015	
6-8	Edoe	Maphumulo (Lina Gayani)	6-8	South Africa	KwaZulu-Natal	Maphumulo	Maphumulo	-29.015	
7-2	Edoe	Warwick market	7-2	South Africa	KwaZulu-Natal	Durban Metro	Durban	-29.85	
7-6	Edoe	Warwick market	7-6	South Africa	KwaZulu-Natal	Durban Metro	Durban	-29.85	
7-9	Edoe	Warwick market	7-9	South Africa	KwaZulu-Natal	Durban Metro	Durban	-29.85	
7-11	Edoe	Warwick market	7-11	South Africa	KwaZulu-Natal	Durban Metro	Durban	-29.85	
7-12	Edoe	Warwick market	7-12	South Africa	KwaZulu-Natal	Durban Metro	Durban	-29.85	
8-1	Edoe	Mkuze market (Agnes)	8-1	South Africa	KwaZulu-Natal	Mkuze	Mkuze	-27.6	
8-2	Edoe	Mkuze market (Agnes)	8-2	South Africa	KwaZulu-Natal	Mkuze	Mkuze	-27.6	
8-3	Edoe	Mkuze market (Agnes)	8-3	South Africa	KwaZulu-Natal	Mkuze	Mkuze	-27.6	
8-4	Edoe	Mkuze market (Agnes)	8-4	South Africa	KwaZulu-Natal	Mkuze	Mkuze	-27.6	
9-1	Edoe	Jozini - Zulu type	9-1	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-2	Edoe	Jozini - Zulu type	9-2	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-3	Edoe	Jozini - Zulu type	9-3	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-4	Edoe	Jozini - Zulu type	9-4	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-5	Edoe	Jozini - Zulu type	9-5	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-7	Edoe	Jozini - Zulu type	9-7	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-8	Edoe	Jozini - Zulu type	9-8	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-11	Edoe	Jozini - Zulu type	9-11	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-13	Edoe	Jozini - Zulu type	9-13	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	

ARC ID	Subspecies	ame	Collectors no	Country of collection	Province/State	District	Nearest Town/village	Latitude	I
9-14	Edoe	Jozini - Zulu type	9-14	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
9-15	Edoe	Jozini - Zulu type	9-15	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
10-1	Edoe	Mbazwana Market #2	10-1	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-2	Edoe	Mbazwana Market #3	10-2	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-3	Edoe	Mbazwana Market #4	10-3	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-4	Edoe	Mbazwana Market #5	10-4	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-6	Edoe	Mbazwana Market #7	10-6	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-7	Edoe	Mbazwana Market #8	10-7	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-8	Edoe	Mbazwana Market #9	10-8	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-9	Edoe	Mbazwana Market #10	10-9	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-10	Edoe	Mbazwana Market #11	10-10	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
10-12	Edoe	Mbazwana Market #12	10-12	South Africa	KwaZulu-Natal	Mbazwana	Mbazwana	-27.466	
11	Edoe	59 in field multiplacation	11	South Africa	KwaZulu-Natal				
12	Edoe	70 in field multiplacation	12	South Africa	KwaZulu-Natal				
13	Edoe	1991 in field multiplacation	13	South Africa	KwaZulu-Natal				
14	Edoe	2053 in field multiplacation	14	South Africa	KwaZulu-Natal				
15	Edoe	2914 in field multiplacation	15	South Africa	KwaZulu-Natal	Umhlubuyalingana	Jozini		
16	Edoe	2919 in field multiplacation	16	South Africa	KwaZulu-Natal	Umhlubuyalingana	Jozini		
17-1	Edoe	3053 in field multiplacation	17-1	South Africa	KwaZulu-Natal	Mthonjaneni	Empangeni		
17-2	Edoe	3053Amzam in field multiplacation	17-2	South Africa	KwaZulu-Natal	Mthonjaneni	Empangeni		
17-3	Edoe	3053ex in field multiplacation	17-3	South Africa	KwaZulu-Natal	Mthonjaneni	Empangeni		
17-4	Edoe	3053/5118 Amzam 4 in field multiplacation	17-4	South Africa	Unknown				
18-1	Edoe	5118 in field multiplacation	18-1	South Africa	KwaZulu-Natal				
18-2	Edoe	5118 Amzam in field multiplacation	18-2	South Africa	KwaZulu-Natal				
19	Edoe	2053ex in field multiplacation	19	South Africa	KwaZulu-Natal	Mthonjaneni	Empangeni		
20	Edoe	Ede Ocha in field multiplacation	20	South Africa	KwaZulu-Natal				
25-1	Edoe	Makatini flats – Mpondo	25-1	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
25-2	Edoe	Makatini flats – Mpondo	25-2	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
26	Edoe	1197	26	South Africa	Eastern Province	Mbhashe	Willowvale	-32.2097	
27	Edoe	1329	27	South Africa	Eastern Province	Ingquza	Lusikisiki	-31.3378	
28	Edoe	1338	28	South Africa	Eastern Province	Ingquza	Lusikisiki	-31.32	
29	Edoe	1637	29	South Africa	Eastern Province	Mbizana	Bizana	-30.8803	

ARC ID	Subspecies	ame	Collectors no	Country of collection	Province/State	District	Nearest Town/village	Latitude	I
30	Edoe	1739	30	South Africa	Eastern Province	OR Thambo	Ngqeleni	-31.66	
31	Edoe	1811	31	South Africa	KwaZulu-Natal		Creighton	-30.0339	
32	Edoe	1862	32	South Africa	KwaZulu-Natal	Durban Metro	Isiphingo	-29.96	
33	Edoe	1865	33	South Africa	KwaZulu-Natal	Durban Metro	Isiphingo	-29.96	
34	Edoe	1866	34	South Africa	KwaZulu-Natal	Durban Metro	Isiphingo	-29.96	
35	Edoe	1889	35	South Africa	KwaZulu-Natal		Umbumbulu	-29.92	
36	Edoe	1906	36	South Africa	KwaZulu-Natal	Camperdown	Pietermaritzbug	-29.56	
37	Edoe	1991	37	South Africa	KwaZulu-Natal	Umbumbulu	Umbumbulu	-29.92	
38	Edoe	2045	38	South Africa	KwaZulu-Natal	Eshowe	Eshowe	-28.8653	
39	Edoe	2073	39	South Africa	KwaZulu-Natal	Eshwoe	Eshowe	-28.8978	
40	Edoe	2119	40	South Africa	KwaZulu-Natal	Eshowe	Eshowe	-28.9106	
41	Edoe	2304	41	South Africa	Mpumalanga	Mbombela	Nelspruit	-25.0944	
42	Edoe	2823	42	South Africa	KwaZulu-Natal	Umhlubuyalingana	Mangozi	-26.95	
43	Edoe	2825	43	South Africa	KwaZulu-Natal	Umhlubuyalingana	Pietermaritzbug	-26.95	
44	Edoe	2914	44	South Africa	KwaZulu-Natal	Umhlubuyalingana	Jozini	-27.416	
45	Edoe	3053	45	South Africa	KwaZulu-Natal	Mthonjaneni	Empangeni	-28.72	
46	Edoe	Brits Pick and Pay	46	South Africa	KwaZulu-Natal	Brits	Brits	-25.616	
47	Edoe	Vilieria Fruit and Veg City	47	South Africa	KwaZulu-Natal	Tshwane	Tshwane	-25.716	
48	Edoe	Makatini RS	48	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
49	Edoe	Makatini dist 6	49	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
50	Edoe	Royal Natal Agric Show	50	South Africa	KwaZulu-Natal	Pietermaritzburg	Pietermaritzburg	-29.583	
51	Edoe	Soshanguve 1	51	South Africa	KwaZulu-Natal	Tshwane	Tshwane	-25.533	
52	Edoe	Soshanguve 2	52	South Africa	KwaZulu-Natal	Tshwane	Tshwane	-25.533	
53	Edoe	Umbumbulu 1	53	South Africa	KwaZulu-Natal	Umbumbulu	Umbumbulu	-29.98	
54	Edoe	Umbumbulu 2	54	South Africa	KwaZulu-Natal	Umbumbulu	Umbumbulu	-29.98	
55	Edoe	Albert Modi 1	55	South Africa	KwaZulu-Natal	Pietermaritzburg	Pietermaritzburg	-29.616	
56	Edoe	Albert Modi 2	56	South Africa	KwaZulu-Natal	Pietermaritzburg	Pietermaritzburg	-29.616	
57	Edoe	Pieter Maritz	57	South Africa	KwaZulu-Natal				
58	Edoe	Cocoindia	58	Nigeria	*	*	*	*	*
64	Edoe	Mtwalume 1	64	South Africa	KwaZulu-Natal	Mtwalume	Mtwalume	-30.5	
65	Edoe	Mtwalume 2	65	South Africa	KwaZulu-Natal	Mtwalume	Mtwalume	-30.5	
66	Edoe	Makatini Mpondo	66	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	

ARC ID	Subspecies	ame	Collectors no	Country of collection	Province/State	District	Nearest Town/village	Latitude	I
67	Edoe	Makatini Round	67	South Africa	KwaZulu-Natal	Jozini	Jozini	-27.416	
68	Edoe	Maphumulo 07	68	South Africa	KwaZulu-Natal	Maphumulo	Maphumulo	-29.15	
71		Dumkehle	71	South Africa	KwaZulu-Natal				
72		SP1	72	Vanuatu					
73		Vaunuatu1	73	Vanuatu					
74		Vaunuatu2	74	Vanuatu					
75		Vaunuatu3	75	Vanuatu					
76		Vaunuatu4	76	Vanuatu					
77		Vaunuatu5	77	Vanuatu					
78		Vaunuatu6	78	Vanuatu					
79		Vaunuatu7	79	Vanuatu					
80		Vaunuatu8	80	Vanuatu					
82		2000-21	BL/HW/05	Hawaii					
83		BC99-11	BL/HW/26	Hawaii					
84		Samoa43	BL/SM/43	Samoa					
85		Alafua	BL/SM/80	Samoa					
86		C3-12	BL/PNG/10	Papua New Guinea					
87		C3-44	BL/PNG/12	Papua New Guinea					
88		Pauli	BL/SM/111	Samoa					
89		Manu	BL/SM/116	Samoa					
90		Manono	BL/SM/120	Samoa					
91		Nu'utele 2	BL/SM/128	Samoa					
92		Fanuatapu	BL/SM/132	Samoa					
93		Malaela 2	BL/SM/148	Samoa					
94		Lepa	BL/SM/149	Samoa					
95		Letogo	BL/SM/151	Samoa					
96		Saleapaga	BL/SM/152	Samoa					
97		Malae-o-le-la	BL/SM/157	Samoa					
98		IND 237	CE/IND/12	Indonesia					
99		IND 231	CE/IND/32	Indonesia					
100		Segamat	CE/MAL/07	Malaysia					
101		Klang	CE/MAL/12	Malaysia					

ARC ID	Subspecies	ame	Collectors no	Country of collection	Province/State	District	Nearest Town/village	Latitude	I
102		Kluang	CE/MAL/14	Malaysia					
103	Edoe	Srisamrong	CE/THA/07	Thailand					
104		Ta Daeng	CE/THA/09	Thailand					
105		Chom tim	CE/VEN/01	Vietnam					
106		Shogatsu-imo	CA/JP/04	Japan					
107		PE x PH15-6	BL/HW/08	Hawaii					
108		Pa'akala	BL/HW/37	Hawaii					
109		C2-E3	BL/PNG/03	Papua New Guinea					
110		C2-E11	BL/PNG/08	Papua New Guinea					
111		C3-22	BL/PNG/11	Papua New Guinea					
112		Samoana	BL/SM/83	Samoa					
113		Tolo-gataua	BL/SM/104	Samoa					
114		Sapapalii	BL/SM/134	Samoa					
115		Matautu	BL/SM/136	Samoa					
116		Vaimauga	BL/SM/143	Samoa					
117		Lalomanu	BL/SM/158	Samoa					
118		IND 155	CE/IND/06	Indonesia					
119		IND 178	CE/IND/08	Indonesia					
120		IND 225	CE/IND/10	Indonesia					
121		Lamputara	CE/IND/14	Indonesia					
122		Apu	CE/IND/20	Indonesia					
123		IND 512	CE/IND/24	Indonesia					
124		Manokwari	CE/IND/31	Indonesia					
125		Phuek	CE/THA/01	Thailand					
126		Surin	CE/THA/02	Thailand					
127		Tha-u-then	CE/THA/19	Thailand					
128		Boklua	CE/THA/24	Thailand					
129		Sangkom	CE/THA/30	Thailand					
130		Tsuronoko	CA/JP/01	Japan					
131		Miyako	CA/JP/03	Japan					

Appendix 2: Taro descriptors (Singh *et al* 2008).
Accession:

Date:

Place:

10 Quantitative measures (average of three individuals):				
1. Number of cormels (CMN)				
2. Weight of cormels (CMW)				
3. Corm length (COL)				
4. Corm breadth (COB)				
5. Corm weight (COW)				
6. Leaf length (LLE)				
7. Leaf width (LWI)				
8. Plant height (PHT)				
9. Number of stolons (STN)				
10. Number of suckers (SUN)				

20 qualitative characteristics

11. **Colour of leaf blade variegation (CBV)**
- Absent
- Yellow
- Green
- Dark green
- Pink
- Red
- Purple
- Black

12. **Corm cortex colour (CCC)**
- White
- Yellow
- Red
- Pink
- Purple
- Other

13. **Corm flesh colour (CFL)**
- White
- Yellow
- Orange
- Pink
- Red
- Red-purple
- Purple
- Other

14. **Corm fibre colour (CFI),**
- White
- Light yellow
- Yellow
- Brown
- Purple
- Other

15. **Corm shape (COS)**
- Conical
- Round
- Cylindrical
- Elliptical
- Dumble
- Elongated
- Clustered

16. **Leaf blade colour (LBC)**
- Yellow
- Green
- Dark green
- Pink
- Other

17. **Leaf blade colour variegation (LBV)**
- Absent
- Present

18. **Predominant position of leaf lamina surface (LPO)**
- Drooping
- Horizontal
- Cup
- Erect apex up
- Erect apex down

19. **Leaf main vein colour (LVC)**
- White
- Yellow
- Orange
- Green
- Pink
- Purple
- Other

20. **Leaf vein pattern (LVP)**
- Y pattern
- I pattern
- V pattern
- Extending
- Other

21. **Petiole basal ring colour (PBC)**
- White
- Green
- Red
- Purple
- Pink

22. **Petiole junction colour (PJC)**
- Absent
- Yellow
- Green
- Red
- Purple
- Other

23. **Petiole junction pattern (PJP)**
- Absent
- Very small
- Small
- Medium
- Large

24. **Petiole lower colour (PLC)**
- White
- Yellow
- Orange
- Light green
- Green
- Red
- Brown
- Purple
- Other

25. **Presence of petiole stripe (PPS)**
- Absent
- Present

26. **Petiole stripe colour (PSC)**
- Absent
- White
- Yellow
- Orange
- Light green
- Other
- Green
- Red
- Brown
- Purple

27. Petiole top colour (PTC)
- White
- Yellow
- Light green
- Green
- Red
- Brown
- Purple
- Other

28. **Type of leaf blade variegation (TBV)**
- Absent
- Mottle
- Fleck
- Stripe

29. **Taro leaf blight resistance (TLB)**
- Very low
- Intermediate
- High
- Very high
- Unknown

30. **Flower formation (FFT)**

1. No flower
2. <10%
3. >10%

Appendix 3: ANOVA Tables

1. Mean Leaf length:

Table 1: ANOVA table for the mean leaf lengths of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	286.878	143.439	9.88	<.0001
Rep(Loc)	6	176.989	29.498	2.03	0.0640
Line	28	1358.126	48.504	3.34	<.0001
Loc*Line	56	1649.352	29.452	2.03	0.0003

Table 2: The t-grouping for mean leaf length for the different localities. The critical value of t = 1.97427 and LSD = 1.1426

Loc	Mean	Std Deviation	N	t Grouping
Roodeplaat	32.321	3.626	87	A
OSCA	31.840	7.181	86	A
Umbumbulu	29.895	1.042	87	B

Table 3: The t-grouping for leaf length. The critical Value of t = 1.97427 and LSD = 3.5533

Line	Mean	Std Dev	N	t Grouping							
BongiweMkhize	34.388	4.355	9	A							
Mbili	34.321	6.088	9	A							
Ngubane	34.038	3.922	9	A							
Thandizwe43	33.887	4.279	9	A							
DlomoDlomo171	33.481	3.609	9	A	B						
Dlomodlomo19	33.408	3.256	9	A	B						
Amzam174	33.289	3.524	9	A	B	C					
Amzam182	32.964	5.375	9	A	B	C	D				
Mabhida	32.920	5.308	9	A	B	C	D				
Gumede	32.683	4.944	9	A	B	C	D				
Nkangala15	32.626	2.927	9	A	B	C	D				
Vilieria47	32.343	3.762	8	A	B	C	D				
Nkangala16	32.327	4.617	9	A	B	C	D				
LungelephiMkhize	31.952	3.549	9	A	B	C	D	E			
Bhengu	31.562	6.154	9	A	B	C	D	E	F		
DlomoDlomo45	31.514	5.026	9	A	B	C	D	E	F		
BusisiweMkhize	31.463	5.273	9	A	B	C	D	E	F		
Nkangala44	31.426	3.013	9	A	B	C	D	E	F		
Modi2	31.117	1.610	9	A	B	C	D	E	F		
Ocha	31.042	3.319	9	A	B	C	D	E	F		
DlomoDlomo14	30.118	4.752	9		B	C	D	E	F	G	
Mhlongo	29.833	1.942	9			C	D	E	F	G	
Dlomodlomo173	29.663	4.934	9				D	E	F	G	
Nxele	29.491	4.681	9				D	E	F	G	
Warwick72	28.731	4.982	9					E	F	G	
Msomi	28.560	3.282	9					E	F	G	
Maphumulo4	28.394	2.914	9						F	G	H
Maphumulo68	26.828	4.257	9							G	H
Klang	24.916	7.542	9								H

2. Leaf width:

Table 4: ANOVA table for the mean leaf width of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	727.311	363.655	27.07	<.0001
Rep(Loc)	6	311.571	51.928	3.87	0.0012
Line	28	1124.688	40.167	2.99	<.0001
Loc*Line	56	1083.306	19.344	1.44	0.0399

Table 5: The t-grouping for mean leaf width in the different localities. The critical value of t = 1.97419 and the LSD = 1.097

Loc	Mean	Std Dev	N	t Grouping
OSCA	27.8310	5.649	87	A
Roodeplaat	24.6102	3.155	87	B
Umbumbulu	24.0390	3.696	87	B

Table 6: The t-grouping for mean leaf width for the different lines. Critical Value of t = 1.97419 and LSD = 3.4108. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t-grouping								
Ngubane	28.798	3.441	9	A								
Mbili	28.554	5.329	9	A	B							
Mabhida	28.398	4.247	9	A	B							
Amzam174	28.338	4.157	9	A	B							
Vilieria47	27.946	5.096	9	A	B	C						
Thandizwe43	27.870	4.019	9	A	B	C						
Gumede	27.499	4.861	9	A	B	C	D					
LungelephiMkhize	27.240	4.570	9	A	B	C	D	E				
BongiweMkhize	27.054	3.938	9	A	B	C	D	E				
Bhengu	26.304	4.437	9	A	B	C	D	E	F			
Nkangala15	26.010	3.233	9	A	B	C	D	E	F	G		
DlomoDlomo171	25.648	5.302	9	A	B	C	D	E	F	G		
BusisiweMkhize	25.537	5.634	9	A	B	C	D	E	F	G		
Dlomodlomo19	25.410	6.371	9	A	B	C	D	E	F	G		
Mhlongo	25.233	3.497	9		B	C	D	E	F	G		
Msomi	25.170	4.174	9		B	C	D	E	F	G		
Nkangala44	24.922	3.822	9			C	D	E	F	G		
Amzam182	24.796	4.867	9			C	D	E	F	G		
DlomoDlomo14	24.774	4.475	9			C	D	E	F	G		
Modi2	24.644	3.634	9			C	D	E	F	G	H	
Warwick72	24.591	2.557	9			C	D	E	F	G	H	
Nkangala16	24.424	5.251	9				D	E	F	G	H	
Ocha	24.264	4.853	9				D	E	F	G	H	
Nxele	24.044	4.110	9					E	F	G	H	
DlomoDlomo45	24.037	3.812	9					E	F	G	H	
Dlomodlomo173	23.497	3.747	9						F	G	H	I
Maphumulo4	22.680	1.759	9							G	H	I
Maphumulo68	21.256	2.540	9								H	I
Klang	20.370	5.021	9									I

3. Leaf Number:

Table 7: ANOVA table for the mean leaf number of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	52703.288	26351.644	559.27	<.0001
Rep(Loc)	6	766.670	127.778	2.71	0.0154
Line	28	3779.064	134.966	2.86	<.0001
Loc*Line	56	3828.476	68.365	1.45	0.0369

Table 8: The t-grouping for mean leaf number in the different localities. The critical value of t = 1.97419 and LSD = 2.0546

Loc	Mean	Std Dev	N	t Grouping
Roodeplaat	44.716	9.232	87	A
OSCA	24.775	9.726	87	B
Umbumbulu	10.039	3.092	87	C

Tabel 9: The t-grouping for mean leaf width for the different lines. Critical Value of t = 1.97419 and LSD = 6.388. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping										
Amzam174	34.297	21.730	9	A										
Thandizwe43	33.073	20.099	9	A	B									
Nkangala15	30.740	19.690	9	A	B	C								
Dlomodlomo19	30.558	19.288	9	A	B	C	D							
Amzam182	30.370	17.961	9	A	B	C	D	E						
Gumede	30.223	18.993	9	A	B	C	D	E	F					
Ngubane	30.150	16.777	9	A	B	C	D	E	F					
Bhengu	30.149	18.951	9	A	B	C	D	E	F					
BusisiweMkhize	29.481	17.077	9	A	B	C	D	E	F	G				
LungelephiMkhize	28.926	16.560	9	A	B	C	D	E	F	G	H			
Ocha	28.592	17.414	9	A	B	C	D	E	F	G	H			
Mabhida	27.333	15.323	9		B	C	D	E	F	G	H			
Modi2	27.259	17.854	9		B	C	D	E	F	G	H	I		
Mhlongo	26.628	16.958	9			C	D	E	F	G	H	I	J	
Mbili	26.221	16.759	9			C	D	E	F	G	H	I	J	K
Vilieria47	25.644	14.459	9			C	D	E	F	G	H	I	J	K
DlomoDlomo171	25.444	18.482	9			C	D	E	F	G	H	I	J	K
BongiweMkhize	25.370	14.901	9			C	D	E	F	G	H	I	J	K
Nkangala44	24.703	13.691	9			C	D	E	F	G	H	I	J	K
Klang	24.260	13.538	9				D	E	F	G	H	I	J	K
DlomoDlomo14	24.038	15.683	9					E	F	G	H	I	J	K
Warwick72	23.890	18.319	9						F	G	H	I	J	K
Msomi	23.851	13.957	9						F	G	H	I	J	K
DlomoDlomo45	23.111	18.936	9							G	H	I	J	K
Nkangala16	22.962	13.664	9								H	I	J	K
Dlomodlomo173	20.888	12.977	9									I	J	K
Maphumulo68	20.259	13.804	9										J	K
Maphumulo4	20.221	14.810	9											K
Nxele	20.149	12.552	9											K

4. Plant height:

Table 10: ANOVA table for the mean plant height of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	15100.598	7550.299	48.36	<.0001
Rep(Loc)	6	1258.235	209.705	1.34	0.2407
Line	28	12169.043	434.608	2.78	<.0001
Loc*Line	56	12203.793	217.924	1.40	0.0547

Table 11: The t-grouping for mean plant height in the different localities. Means with the same letter were not significantly different. The critical value of t = 1.97419 and LSD = 3.7401

Loc	Mean	Std Dev	N	t Grouping
OSCA	80.822	20.505	87	A
Roodeplaat	76.799	10.337	87	B
Umbumbulu	63.056	8.7001	87	C

Table 12: The t-grouping for mean plant height for the different lines. Critical Value of t = 1.97419 and LSD = 11.629. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping							
Ngubane	85.631	15.146	9	A							
Amzam174	85.427	13.838	9	A							
Thandizwe43	82.721	12.298	9	A	B						
BongiweMkhize	81.444	19.136	9	A	B	C					
Vilieria47	79.446	14.633	9	A	B	C	D				
Mbili	79.112	15.641	9	A	B	C	D	E			
Nkangala15	79.088	12.415	9	A	B	C	D	E			
Nkangala16	78.092	18.118	9	A	B	C	D	E			
LungelephiMkhize	77.852	15.405	9	A	B	C	D	E			
DlomoDlomo171	77.740	18.737	9	A	B	C	D	E			
Gumede	76.408	17.881	9	A	B	C	D	E	F		
Nkangala44	76.168	11.597	9	A	B	C	D	E	F		
Dlomodlomo19	75.647	18.818	9	A	B	C	D	E	F		
Mabhida	75.408	11.221	9	A	B	C	D	E	F		
Amzam182	74.943	21.427	9	A	B	C	D	E	F		
Bhengu	74.666	19.040	9	A	B	C	D	E	F		
Mhlongo	71.684	10.312	9		B	C	D	E	F	G	
Ocha	71.056	14.201	9			C	D	E	F	G	H
BusisiweMkhize	71.019	18.235	9			C	D	E	F	G	H
Modi2	70.686	16.559	9			C	D	E	F	G	H
Msomi	69.767	16.057	9				D	E	F	G	H
Warwick72	69.537	12.268	9				D	E	F	G	H
DlomoDlomo45	68.693	14.753	9				D	E	F	G	H
DlomoDlomo14	67.722	14.066	9					E	F	G	H
Nxele	65.871	14.240	9						F	G	H
Dlomodlomo173	65.563	18.890	9						F	G	H
Maphumulo4	61.220	9.249	9							G	H
Klang	61.148	7.953	9							G	H
Maphumulo68	59.454	13.355	9								H

5. Canopy diameter:

Table 13: ANOVA table for the mean canopy diameter of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	94683.22092	47341.61046	305.43	<.0001
Rep(Loc)	6	2099.72880	349.95480	2.26	0.0403
Line	28	9383.55366	335.12692	2.16	0.0015
Loc*Line	56	8302.47451	148.25847	0.96	0.5660

Table 14: The t-grouping for mean canopy diameter in the different localities. Means with the same letter were not significantly different. Critical Value of t = 1.97427 and LSD = 3.734

Loc	Mean	N	t Grouping
Roodeplaat	100.536	87	A
OSCA	69.494	86	B
Umbumbulu	54.844	87	C

Table 15: The t-grouping for mean canopy diameter for the different lines. Critical Value of t = 1.97427 and LSD = 11.612. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping									
Thandizwe43	86.630	25.265	9	A									
Amzam174	86.187	22.271	9	A	B								
Nkangala44	84.259	21.834	9	A	B	C							
Amzam182	81.480	28.446	9	A	B	C	D						
Dlomodlomo19	80.556	31.528	9	A	B	C	D	E					
DlomoDlomo14	79.297	24.523	9	A	B	C	D	E	F				
Mbili	79.147	23.710	9	A	B	C	D	E	F				
Nkangala16	78.592	22.259	9	A	B	C	D	E	F	G			
DlomoDlomo171	78.147	26.787	9	A	B	C	D	E	F	G	H		
Bhengu	78.073	23.397	9	A	B	C	D	E	F	G	H		
LungelephiMkhize	77.462	18.250	9	A	B	C	D	E	F	G	H		
Ocha	75.519	18.025	9	A	B	C	D	E	F	G	H	I	
Ngubane	75.519	24.896	9	A	B	C	D	E	F	G	H	I	
Nkangala15	75.500	22.183	9	A	B	C	D	E	F	G	H	I	
Vilieria47	75.444	18.113	9	A	B	C	D	E	F	G	H	I	
Mabhida	74.852	22.622	9		B	C	D	E	F	G	H	I	
BongiweMkhize	74.111	20.322	9			C	D	E	F	G	H	I	
Msomi	73.852	18.411	9			C	D	E	F	G	H	I	
DlomoDlomo45	73.688	29.749	9			C	D	E	F	G	H	I	
Gumede	73.667	24.710	9			C	D	E	F	G	H	I	
Modi2	73.037	28.944	9			C	D	E	F	G	H	I	J
BusisiweMkhize	71.554	17.248	9				D	E	F	G	H	I	J
Dlomodlomo173	69.666	23.445	9					E	F	G	H	I	J
Mhlongo	68.333	23.345	9						F	G	H	I	J
Klang	67.852	22.331	9						F	G	H	I	J
Nxele	67.167	23.499	9							G	H	I	J
Warwick72	66.814	30.306	9								H	I	J
Maphumulo4	64.722	22.014	9									I	J
Maphumulo68	61.811	24.110	8										J

6. Number of suckers

Table 16: ANOVA table for the mean number of suckers of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	7271.477870	3635.738935	548.68	<.0001
Rep(Loc)	6	353.423462	58.903910	8.89	<.0001
Line	28	947.411842	33.836137	5.11	<.0001
Loc*Line	56	708.662869	12.654694	1.91	0.0009

Table 17: The t-grouping for mean number of suckers in the different localities. Means with the same letter were not significantly different. Critical Value of t = 1.97427 and LSD = 0.772

Loc	Mean	N	t Grouping
Roodeplaat	16.0543	86	A
OSCA	11.5317	87	B
Umbumbulu	3.2761	87	C

Table 18: The t-grouping for mean number of suckers for the different lines. Critical Value of t = 1.97427 and LSD = 2.4009. Means with the same letter were not significantly different.

Line	Mean	Srd Dev	N	t Grouping										
Amzam174	14.558	7.628	9	A										
Nkangala15	13.518	8.706	9	A	B									
Thandizwe43	13.408	7.759	9	A	B									
DlomoDlomo171	13.074	8.187	9	A	B	C								
Dlomodlomo19	12.184	6.409	9	A	B	C	D							
Amzam182	12.000	6.423	9		B	C	D	E						
Ocha	11.927	7.702	9		B	C	D	E						
Nkangala44	11.371	4.610	9		B	C	D	E	F					
BusisiweMkhize	11.000	7.118	9			C	D	E	F	G				
Modi2	10.999	7.295	9			C	D	E	F	G				
Nkangala16	10.927	5.648	9			C	D	E	F	G				
Ngubane	10.778	6.309	9			C	D	E	F	G	H			
Bhengu	10.519	6.345	9				D	E	F	G	H			
Mbili	10.124	9.751	8				D	E	F	G	H	I		
BongiweMkhize	10.074	6.702	9				D	E	F	G	H	I		
Maphumulo4	10.038	5.096	9				D	E	F	G	H	I		
Dlomodlomo173	9.703	6.402	9					E	F	G	H	I	J	
Mabhida	9.519	5.470	9						F	G	H	I	J	
LungelephiMkhize	9.109	5.865	9						F	G	H	I	J	K
Gumede	9.073	5.213	9						F	G	H	I	J	K
Vilieria47	8.993	3.960	9						F	G	H	I	J	K
Mhlongo	8.779	5.700	9							G	H	I	J	K
DlomoDlomo14	8.666	5.099	9							G	H	I	J	K
DlomoDlomo45	8.442	6.194	9								H	I	J	K
Warwick72	8.429	5.973	9								H	I	J	K
Klang	8.057	5.689	9									I	J	K
Msomi	8.000	5.196	9									I	J	K
Nxele	7.630	4.774	9										J	K
Maphumulo68	6.777	4.812	9											K

7. Number of cormels harvested from a single plant

Table 19: ANOVA table for the mean number of cormels of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	31001.855	15500.927	334.72	<.0001
Rep(Loc)	6	884.555	147.425	3.18	0.0055
Line	28	15354.256	548.366	11.84	<.0001
Loc*Line	56	10443.248	186.486	4.03	<.0001

Table 20: The t-grouping for mean number of cormels harvested per plant in the different localities. Means with the same letter were not significantly different. Critical value of t = 1.97419 and LSD = 2.037.

Loc	Mean	Std Dev	N	t Grouping
Roodeplaat	34.697	16.393	87	A
OSCA	23.691	11.039	87	B
Umbumbulu	8.131	3.1815	87	C

Table 21: The t-grouping for mean number of cormels for the different lines. Critical Value of t = 1.97419 and LSD = 6.3332. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping
Dlomodlomo19	35.111	21.997	9	A
DlomoDlomo171	34.557	24.906	9	A
Amzam174	33.370	17.841	9	A B
Nkangala16	32.443	20.503	9	A B C
Amzam182	31.019	21.727	9	A B C D
DlomoDlomo14	30.038	18.769	9	A B C D E
Thandizwe43	29.297	15.915	9	A B C D E
Ocha	28.149	16.226	9	B C D E
Nkangala15	27.814	17.014	9	B C D E
Nkangala44	26.853	15.212	9	C D E
Modi2	26.482	13.861	9	C D E
DlomoDlomo45	25.556	18.905	9	D E F
Dlomodlomo173	25.222	18.634	9	D E F G
Maphumulo4	24.352	20.579	9	E F G H
Mabhida	19.853	9.504	9	F G H I
Nxele	19.147	15.728	9	G H I
Vilieria47	18.183	9.046	9	H I J
Gumede	17.557	8.042	9	I J K
Mhlongo	17.370	9.156	9	I J K
BongiweMkhize	17.148	12.075	9	I J K
Bhengu	17.074	7.940	9	I J K
BusisiweMkhize	16.851	8.668	9	I J K
Ngubane	16.480	7.854	9	I J K L
Warwick72	16.073	11.158	9	I J K L
LungelephiMkhize	14.722	8.649	9	I J K L
Msomi	12.557	7.091	9	J K L M
Mbili	11.667	5.479	9	K L M
Maphumulo68	10.167	4.626	9	L M
Klang	7.907	4.915	9	M

8. Weight of cormels harvested from a single plant

Table 22: ANOVA table for the mean weight of cormels harvested from a single plant of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	14.680	7.340	150.19	<.0001
Rep(Loc)	6	0.9764	0.162	3.33	0.0040
Line	28	3.3429	0.119	2.44	0.0003
Loc*Line	56	6.1053	0.109	2.23	<.0001
LocxLin	0	0.0000	.	.	.

Table 23: The t-grouping for mean weight of cormels harvested per plant in the different localities. Means with the same letter were not significantly different. Critical Value of t = 1.97419 and LSD = 0.0662

Loc	Mean	Std Dev	N	t Grouping
Roodeplaat	0.84747	0.243	87	A
OSCA	0.79644	0.381	87	A
Umbumbulu	0.32080	0.108	87	B

Table 24: The t-grouping for mean weight of cormels harvested per plant for the different lines. Critical Value of t = 1.97419 and LSD = 0.2057. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping						
Thandizwe43	0.9000	0.466	9	A						
Mabhida	0.8656	0.401	9	A	B					
Amzam174	0.7867	0.343	9	A	B	C				
Ngubane	0.7833	0.512	9	A	B	C				
Gumede	0.7689	0.359	9	A	B	C	D			
BusisiweMkhize	0.7478	0.494	9	A	B	C	D	E		
Mhlongo	0.7333	0.387	9	A	B	C	D	E		
Bhengu	0.7322	0.290	9	A	B	C	D	E		
Dlomodlomo19	0.6944	0.316	9	A	B	C	D	E		
Modi2	0.6878	0.335	9		B	C	D	E		
LungelephiMkhize	0.6856	0.388	9		B	C	D	E		
Ocha	0.6833	0.322	9		B	C	D	E		
DlomoDlomo14	0.6622	0.361	9		B	C	D	E		
Nkangala15	0.6533	0.364	9			C	D	E		
BongiweMkhize	0.6478	0.402	9			C	D	E		
Amzam182	0.6422	0.370	9			C	D	E		
Nkangala16	0.6256	0.283	9			C	D	E		
DlomoDlomo45	0.6200	0.348	9			C	D	E		
Nxele	0.6144	0.337	9			C	D	E		
Vilieria47	0.6111	0.368	9			C	D	E		
Mbili	0.6044	0.330	9			C	D	E	F	
Warwick72	0.5944	0.399	9			C	D	E	F	
DlomoDlomo171	0.5922	0.287	9			C	D	E	F	
Nkangala44	0.5844	0.200	9			C	D	E	F	
Maphumulo4	0.5833	0.367	9			C	D	E	F	
Msomi	0.5756	0.324	9				D	E	F	
Dlomodlomo173	0.5522	0.406	9					E	F	G
Maphumulo68	0.4011	0.217	9						F	G
Klang	0.3589	0.244	9							G

9. Corm length

Table 25: ANOVA table for the mean corm length of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	23514.95350	11757.47675	60.81	<.0001
Rep(Loc)	6	1365.97072	227.66179	1.18	0.3207
Line	28	6812.95758	243.31991	1.26	0.1885
Loc*Line	56	15725.48557	280.81224	1.45	0.0368

Table 26: The t-grouping for mean corm length harvested per plant in the different localities. Means with the same letter were not significantly different. Critical Value of t = 1.97436 and LSD 4.1786

Loc	Mean	N	t Grouping
OSCA	79.018	85	A
Umbumbulu	68.458	87	B
Roodeplaat	55.676	87	C

Table 27: The t-grouping for mean length of cormels harvested per plant for the different lines. Critical Value = 1.97436 and LSD = 12.997. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping		
LungelephiMkhize	82.116	25.190	9	A		
Amzam174	75.660	24.356	9	A	B	
Nxele	75.218	22.890	9	A	B	
Ngubane	72.773	19.735	9	A	B	C
Nkangala16	72.554	24.902	9	A	B	C
Ocha	71.848	15.557	9	A	B	C
Msomi	71.484	26.113	9	A	B	C
Thandizwe43	71.358	16.350	8	A	B	C
Bhengu	71.237	18.028	9	A	B	C
Nkangala15	68.949	24.530	9		B	C
Mhlongo	68.030	10.846	9		B	C
Warwick72	68.023	18.025	9		B	C
Maphumulo68	67.644	14.632	9		B	C
BusisiweMkhize	67.589	20.957	9		B	C
Mbili	67.369	13.431	9		B	C
DlomoDlomo171	67.279	17.004	9		B	C
DlomoDlomo45	67.146	18.495	9		B	C
BongiweMkhize	65.810	14.261	9		B	C
Mabhida	64.673	15.117	8		B	C
Gumede	64.390	11.447	9		B	C
Klang	63.887	6.822	9		B	C
Dlomodlomo19	63.800	14.442	9		B	C
Modi2	63.674	16.264	9		B	C
Amzam182	63.290	12.736	9		B	C
DlomoDlomo14	63.046	10.523	9		B	C
Vilieria47	61.062	13.964	9			C
Maphumulo4	60.766	11.550	9			C
Dlomodlomo173	60.666	9.963	9			C
Nkangala44	60.020	24.346	9			C

10, Corm breadth

Table 28: ANOVA table for the mean corm breath of 29 lines at three different localities

Source	DF	Type I SS	Mean Square	F Value	Pr > F
Loc	2	19932.305	9966.152	79.57	<.0001
Rep(Loc)	6	794.273	132.378	1.06	0.3906
Line	28	5265.994	188.071	1.50	0.0617
Loc*Line	56	8972.745	160.227	1.28	0.1180

Table 29: the t-grouping for mean breadth of cormels harvested per plant in the different localities. Means with the same letter were not significantly different. Critical Value of t = 1.97419 and LSD = 3.3498

Loc	Mean	Std Dev	N	t Grouping
OSCA	57.463	17.294	87	A
Umbumbulu	39.747	7.013	87	B
Roodeplaat	38.199	8.436	87	B

Table 30: The t-grouping for mean breath of corms for the different lines. Critical Value of t = 1.97419 and LSD = 10.415. Means with the same letter were not significantly different.

Line	Mean	Std Dev	N	t Grouping			
Nxele	57.358	20.109	9	A			
Amzam174	52.541	19.819	9	A	B		
Gumede	51.878	15.081	9	A	B		
Nkangala16	50.371	24.596	9	A	B	C	
LungelephiMkhize	50.344	23.168	9	A	B	C	
Thandizwe43	49.964	17.018	9	A	B	C	
Ngubane	48.397	15.720	9	A	B	C	D
Nkangala15	46.460	22.752	9		B	C	D
Bhengu	46.393	11.975	9		B	C	D
Msomi	46.379	22.130	9		B	C	D
Dlomodlomo19	46.308	13.308	9		B	C	D
Mhlongo	45.747	8.196	9		B	C	D
Warwick72	45.511	15.561	9		B	C	D
DlomoDlomo171	44.612	9.457	9		B	C	D
Maphumulo68	44.576	6.088	9		B	C	D
BusisiweMkhize	44.162	17.354	9		B	C	D
Ocha	43.833	8.896	9		B	C	D
Nkangala44	43.710	19.779	9		B	C	D
Mabhida	43.540	9.406	9		B	C	D
Vilieria47	43.421	16.463	9		B	C	D
Mbili	43.081	8.154	9		B	C	D
Klang	42.802	7.570	9		B	C	D
Maphumulo4	41.346	12.963	9			C	D
DlomoDlomo45	40.813	6.073	9			C	D
Modi2	40.560	15.164	9			C	D
Amzam182	39.211	8.048	9				D
BongiweMkhize	38.828	8.141	9				D
DlomoDlomo14	38.733	4.687	9				D
Dlomodlomo173	38.072	5.755	9				D

Appendix 4: AMMI ANOVA tables

Table 1: ANOVA table for AMMI model for leaf length.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	6535	25.14		
Treatments	86	3321	38.61	2.11	<0.001
Genotypes	28	1378	49.21	2.68	<0.001
Environments	2	267	133.50	5.94	0.0032
Block	6	135	22.46	1.23	0.2958
Interactions	56	1676	29.92	1.63	0.0091
IPCA 1	29	1511	52.09	2.84	<0.001
IPCA 2	27	165	6.12	0.33	0.9993
Residuals	0	0			
Error	168	3080	18.33		

Table 2: ANOVA table for AMMI model for leaf width

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	5503	21.17		
Treatments	86	2935	34.13	2.54	<0.001
Genotypes	28	1125	40.17	2.99	<0.001
Environments	2	727	363.66	7.00	0.0012
Block	6	312	51.93	3.87	0.0012
Interactions	56	1083	19.34	1.44	0.0399
IPCA 1	29	848	29.23	2.18	0.0012
IPCA 2	27	236	8.73	0.65	0.9065
Residuals	0	0			
Error	168	2257	13.43		

Table 3: ANOVA table for AMMI model for the number of leaves on a single plant

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	68993	265		
Treatments	86	60311	701	14.88	<0.001
Genotypes	28	3779	135	2.86	<0.001
Environments	2	52703	26352	206.23	<0.001
Block	6	767	128	2.71	0.0154
Interactions	56	3828	68	1.45	0.0369
IPCA 1	29	2434	84	1.78	0.0130
IPCA 2	27	1395	52	1.10	0.3493
Residuals	0	0			
Error	168	7916	47		

Table 4: ANOVA table for AMMI model for plant height.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	66962	258		
Treatments	86	39473	459	2.94	<0.001
Genotypes	28	12169	435	2.78	<0.001
Environments	2	15101	7550	36.00	<0.001
Block	6	1258	210	1.34	0.2407
Interactions	56	12204	218	1.40	0.0547
IPCA 1	29	9668	333	2.14	0.0015
IPCA 2	27	2536	94	0.60	0.9397
Residuals	0	0			
Error	168	26230	156		

Table 5: ANOVA table for AMMI model for canopy diameter.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	141826	545		
Treatments	86	113865	1324	8.54	<0.001
Genotypes	28	10155	363	2.34	<0.001
Environments	2	95082	47541	137.42	<0.001
Block	6	2076	346	2.23	0.0425
Interactions	56	8628	154	0.99	0.4964
IPCA 1	29	5428	187	1.21	0.2289
IPCA 2	27	3200	119	0.76	0.7916
Residuals	0	0			
Error	167	25885	155		

Table 6: ANOVA table for AMMI model for the number of suckers per plant.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	10398	40.0		
Treatments	86	8757	101.8	13.72	<0.001
Genotypes	28	949	33.9	4.57	<0.001
Environments	2	7201	3600.5	54.77	<0.001
Block	6	394	65.7	8.86	<0.001
Interactions	56	607	10.8	1.46	0.0341
IPCA 1	29	336	11.6	1.56	0.0434
IPCA 2	27	271	10.0	1.35	0.1279
Residuals	0	0			
Error	168	1246	7.4		

Table 7: ANOVA table for AMMI model for the number of cormels harvested from the single plant.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	65464	252		
Treatments	86	56799	660	14.26	<0.001
Genotypes	28	15354	548	11.84	<0.001
Environments	2	31002	15501	105.14	<0.001
Block	6	885	147	3.18	0.0055
Interactions	56	10443	186	4.03	<0.001
IPCA 1	29	8332	287	6.20	<0.001
IPCA 2	27	2111	78	1.69	0.0248
Residuals	0	0			
Error	168	7780	46		

Table 8: ANOVA table for AMMI model for the weight of the cormels harvested from a single plant.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	33.315	0.1281		
Treatments	86	24.128	0.2806	5.74	<0.001
Genotypes	28	3.343	0.1194	2.44	<0.001
Environments	2	14.680	7.3400	45.10	<0.001
Block	6	0.976	0.1627	3.33	0.0040
Interactions	56	6.105	0.1090	2.23	<0.001
IPCA 1	29	5.023	0.1732	3.54	<0.001
IPCA 2	27	1.083	0.0401	0.82	0.7209
Residuals	0	0.000			
Error	168	8.211	0.0489		

Table 9: ANOVA table for AMMI model for the length of the corms.

Source	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	80619	310		
Treatments	86	46862	545	2.82	<0.001
Genotypes	28	7079	253	1.31	0.1530
Environments	2	23692	11846	42.75	<0.001
Block	6	1663	277	1.43	0.2047
Interactions	56	16091	287	1.49	0.0287
IPCA 1	29	12231	422	2.18	0.0012
IPCA 2	27	3860	143	0.74	0.8206
Residuals	0	0			
Error	166	32094	193		

Table 10: ANOVA table for AMMI model for the breadth of the corms

	d.f.	s.s.	m.s.	v.r.	F pr
Total	260	56006	215		
Treatments	86	34171	397	3.17	<0.001
Genotypes	28	5266	188	1.50	0.0617
Environments	2	19932	9966	75.29	<0.001
Block	6	794	132	1.06	0.3906
Interactions	56	8973	160	1.28	0.1180
IPCA 1	29	7352	254	2.02	0.0030
IPCA 2	27	1621	60	0.48	0.9866
Residuals	0	0			
Error	168	21041	125		

Appendix 5: AMMI biplots

1. Leaf Length

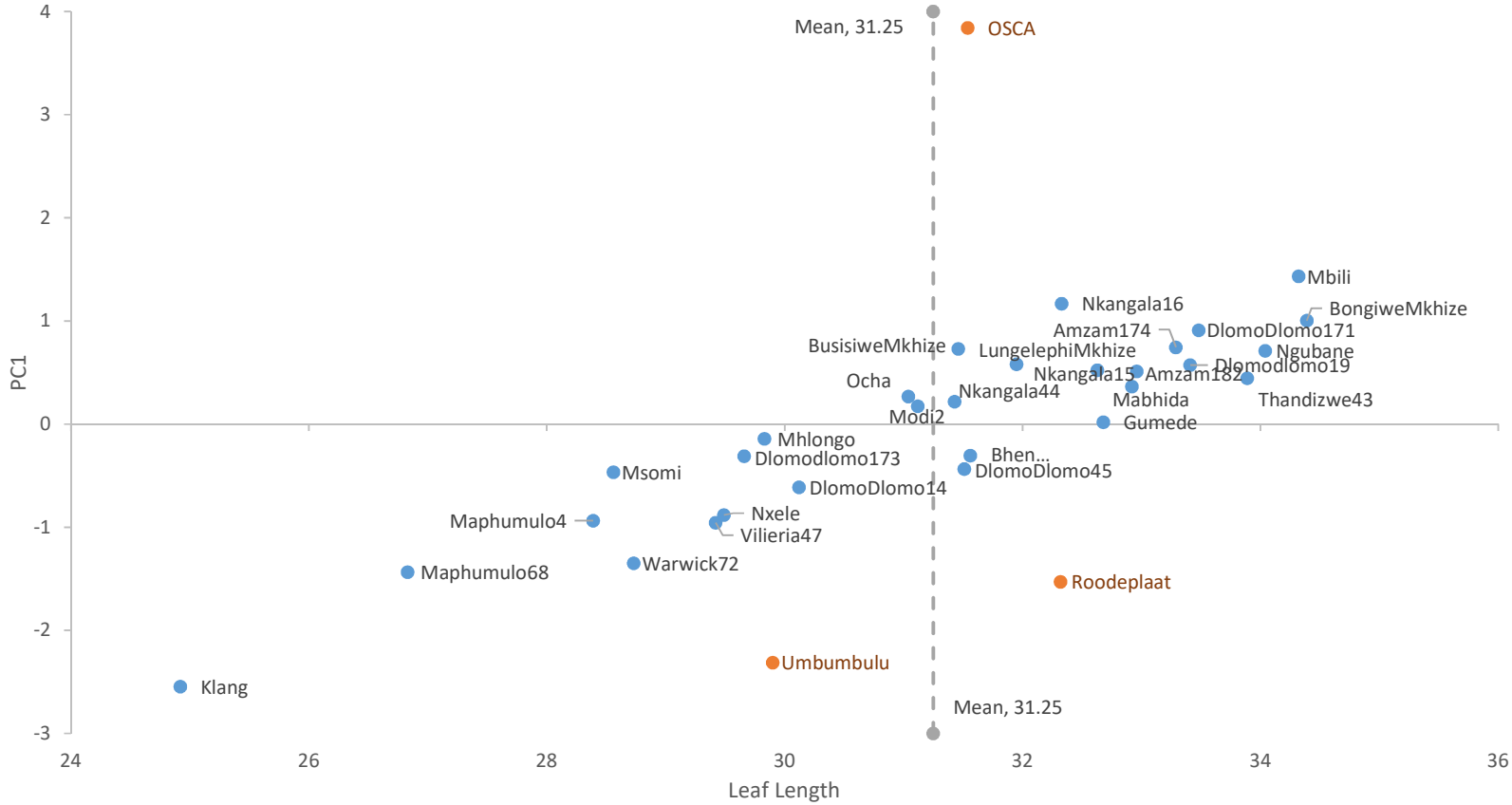

Figure 1: The AMMI1 model for leaf length, plotting the overall mean of each line and locality against the first principal component (PC1).

2. Leaf width

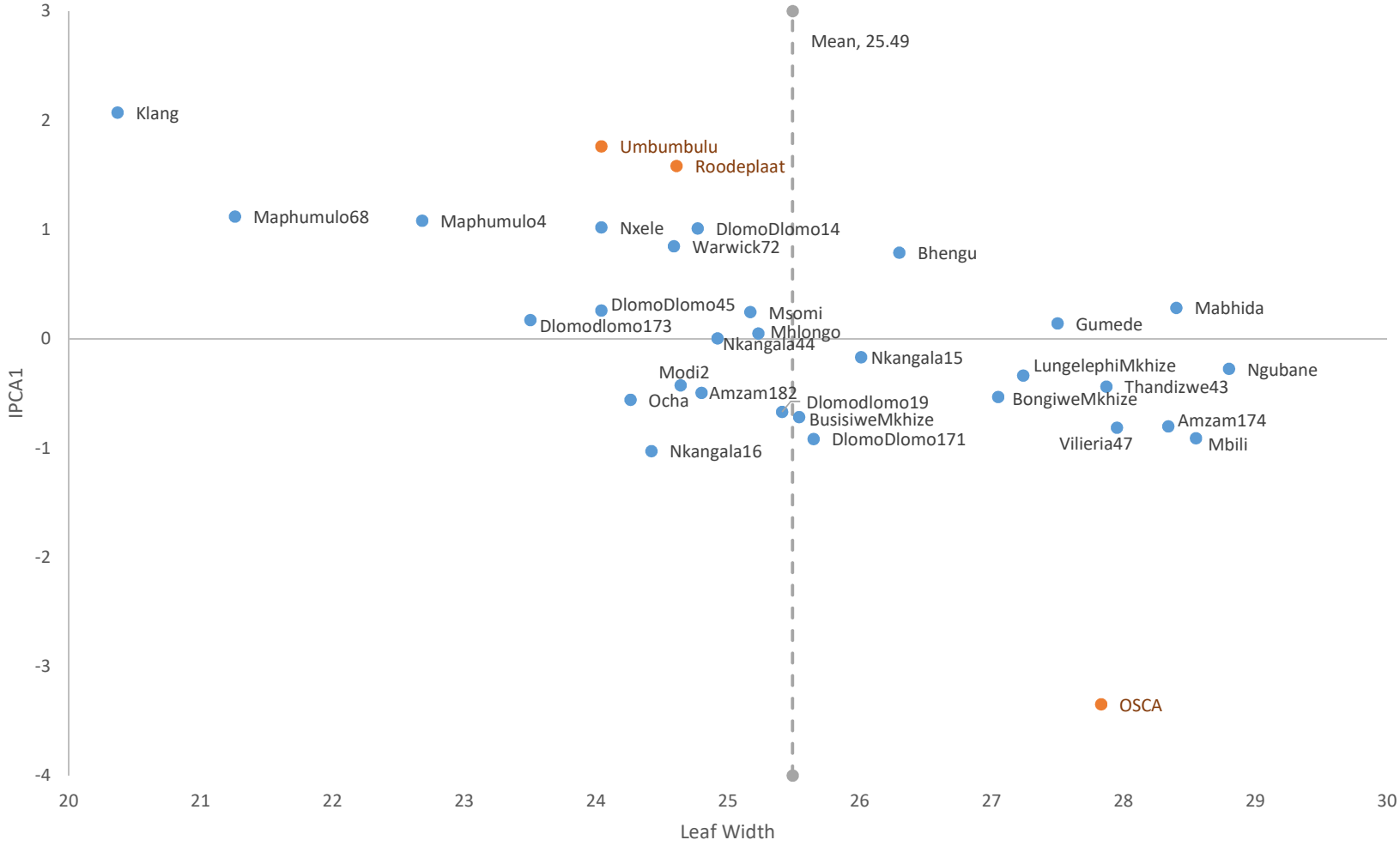

Table 2: The AMMI1 model for leaf width, plotting the overall mean of each line and locality against the first principal component (PC1).

3. Number of leaves on a single plant

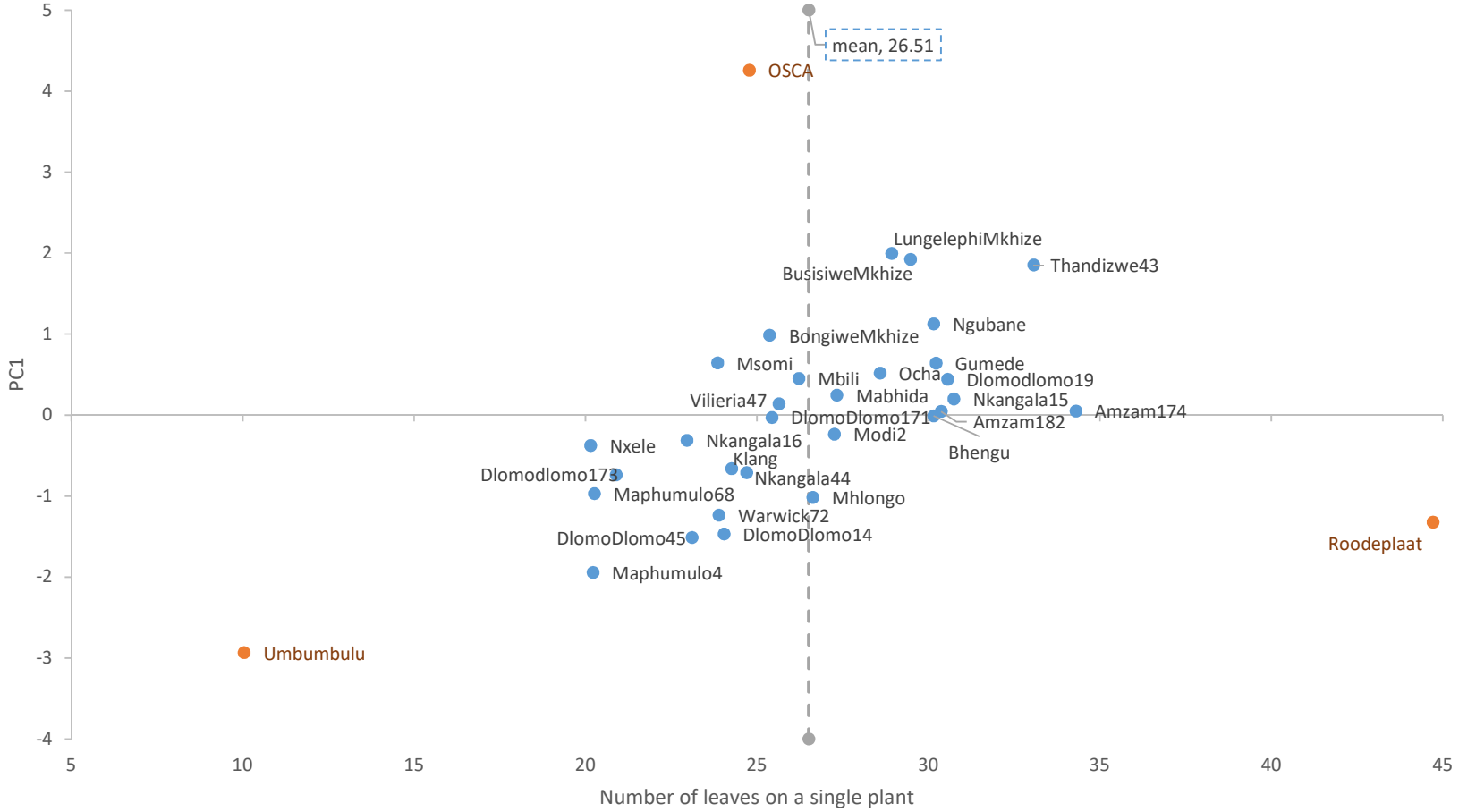

Figure 3: The AMMI1 model for number of leaves on a single plant, plotting the overall mean of each line and locality against the first principal component (PC1).

4. Plant height

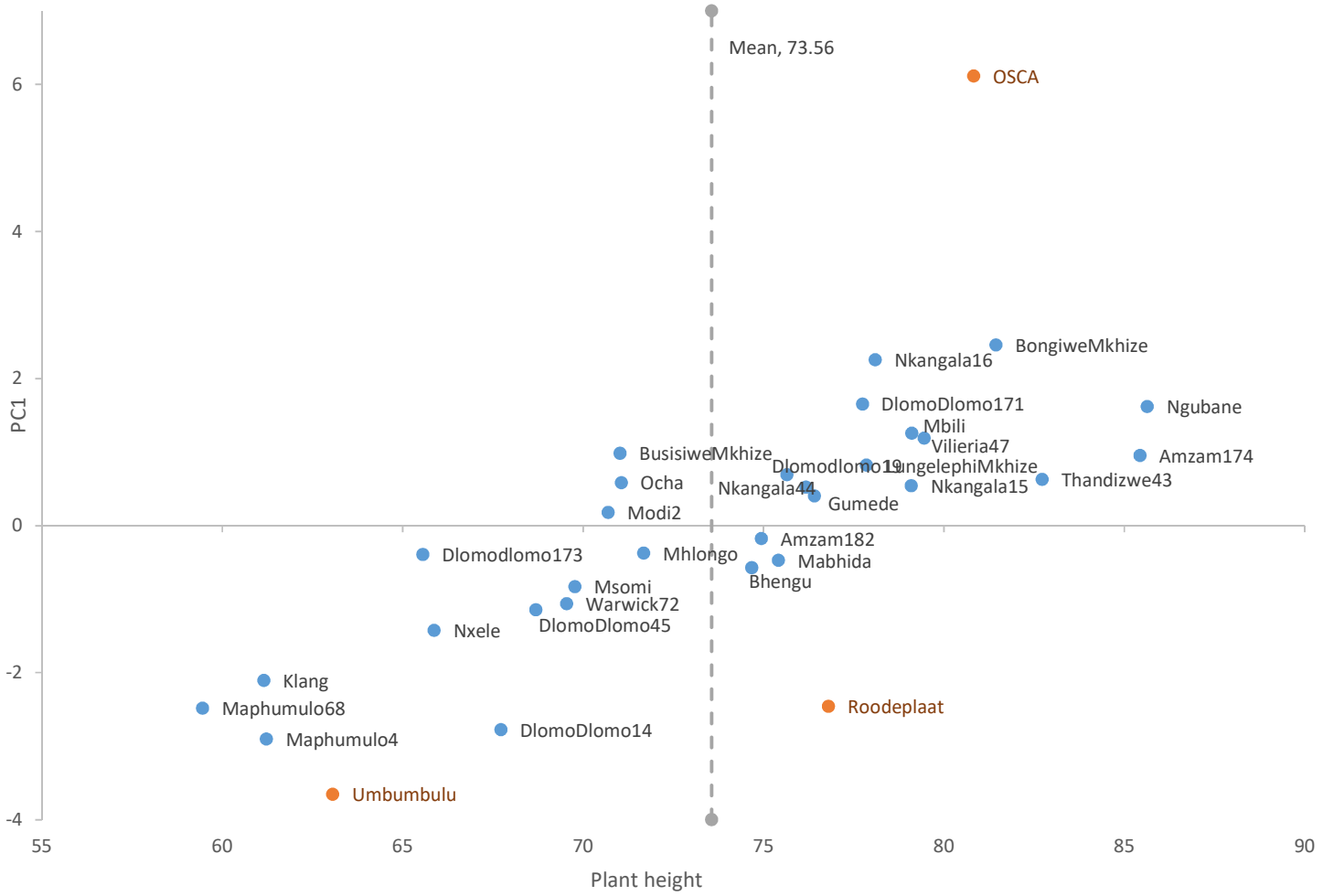

Figure 4: The AMMI1 model for plant height, plotting the overall mean of each line and locality against the first principal component (PC1).

5. Canopy diameter

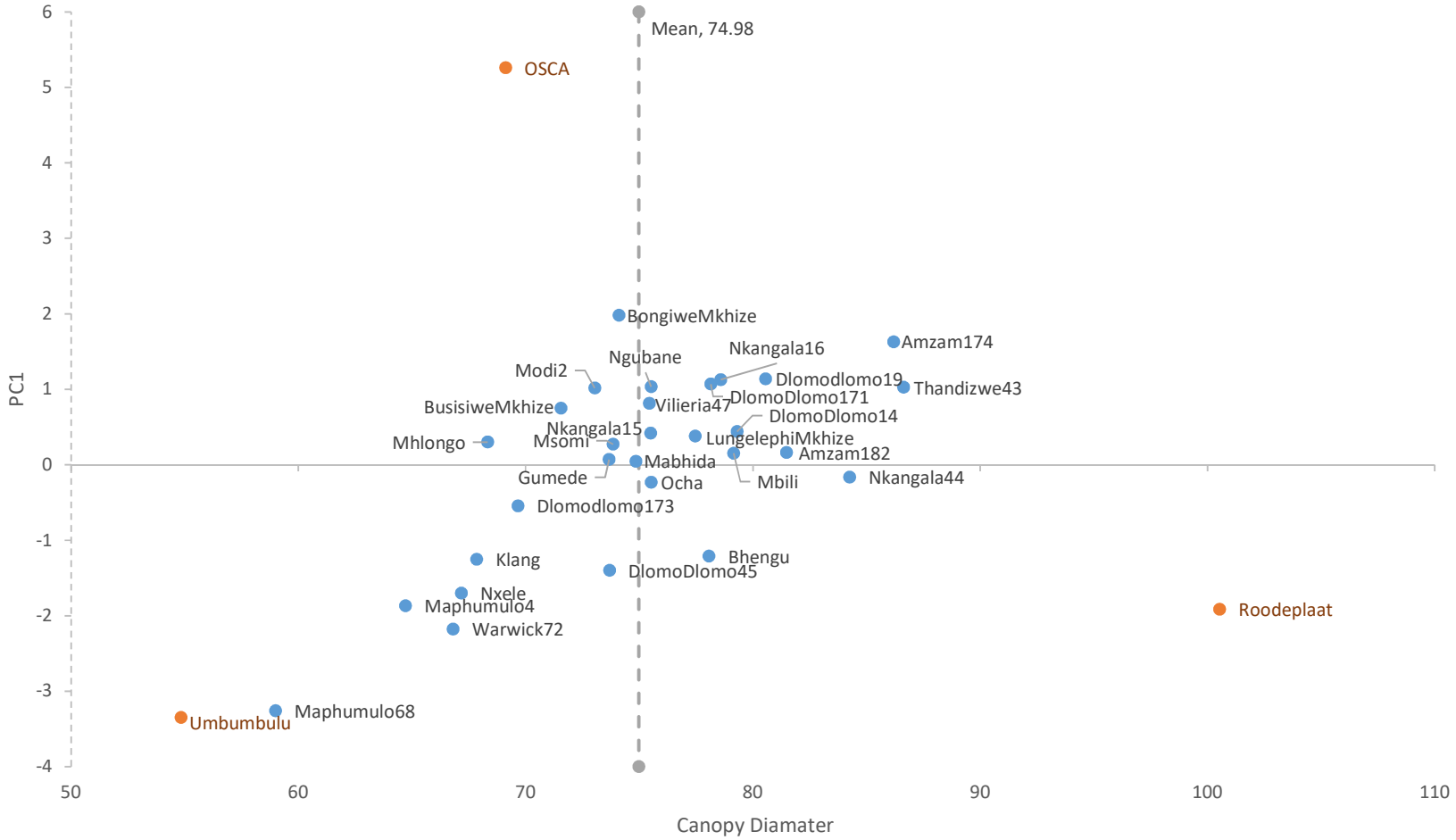

Figure 5: The AMMI1 model for canopy diameter, plotting the overall mean of each line and locality against the first principal component (PC1).

6. Number of suckers

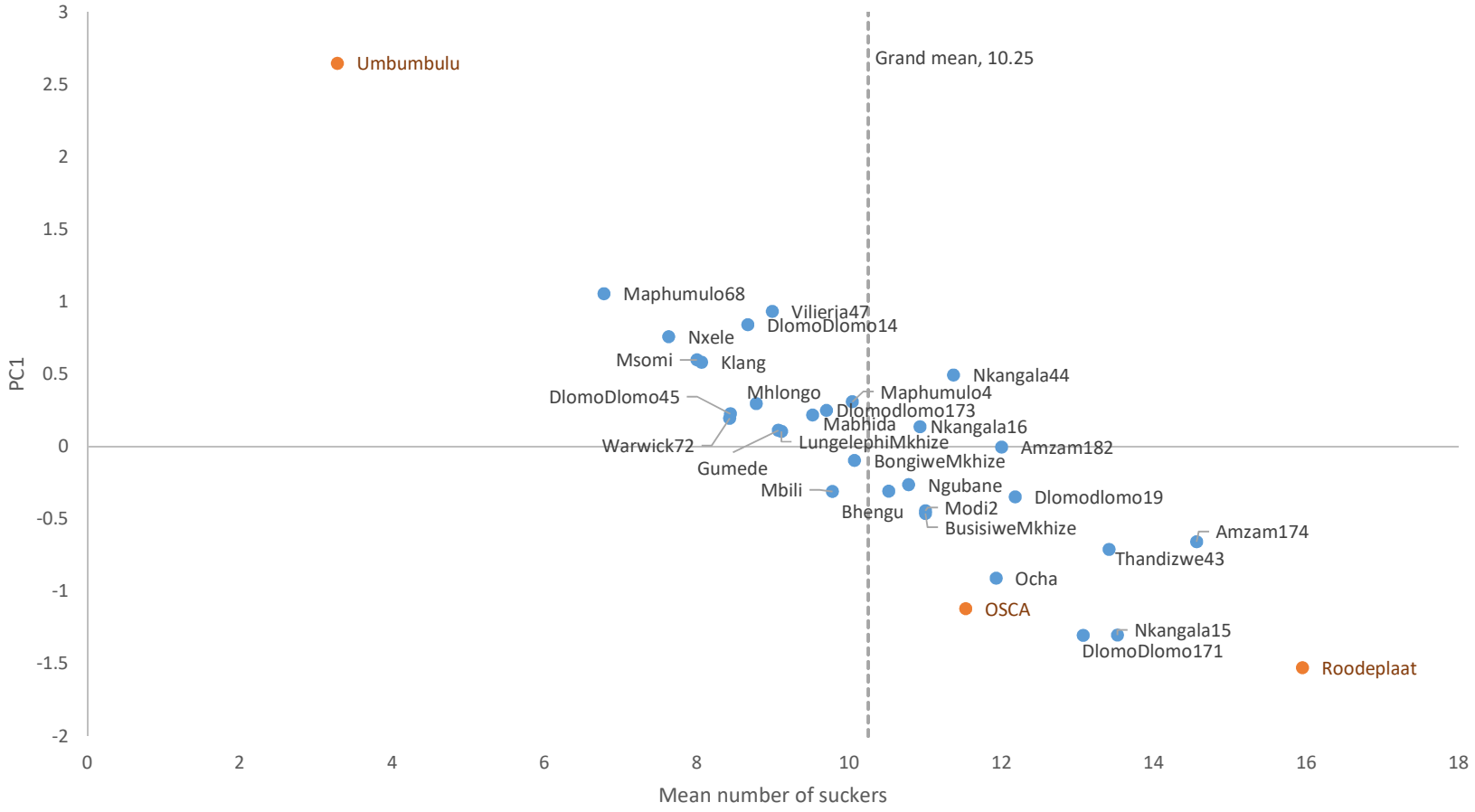

Figure 6: The AMMI1 model for number of suckers, plotting the overall mean of each line and locality against the first principal component (PC1).

7. Number of Cormels harvested from a single plant

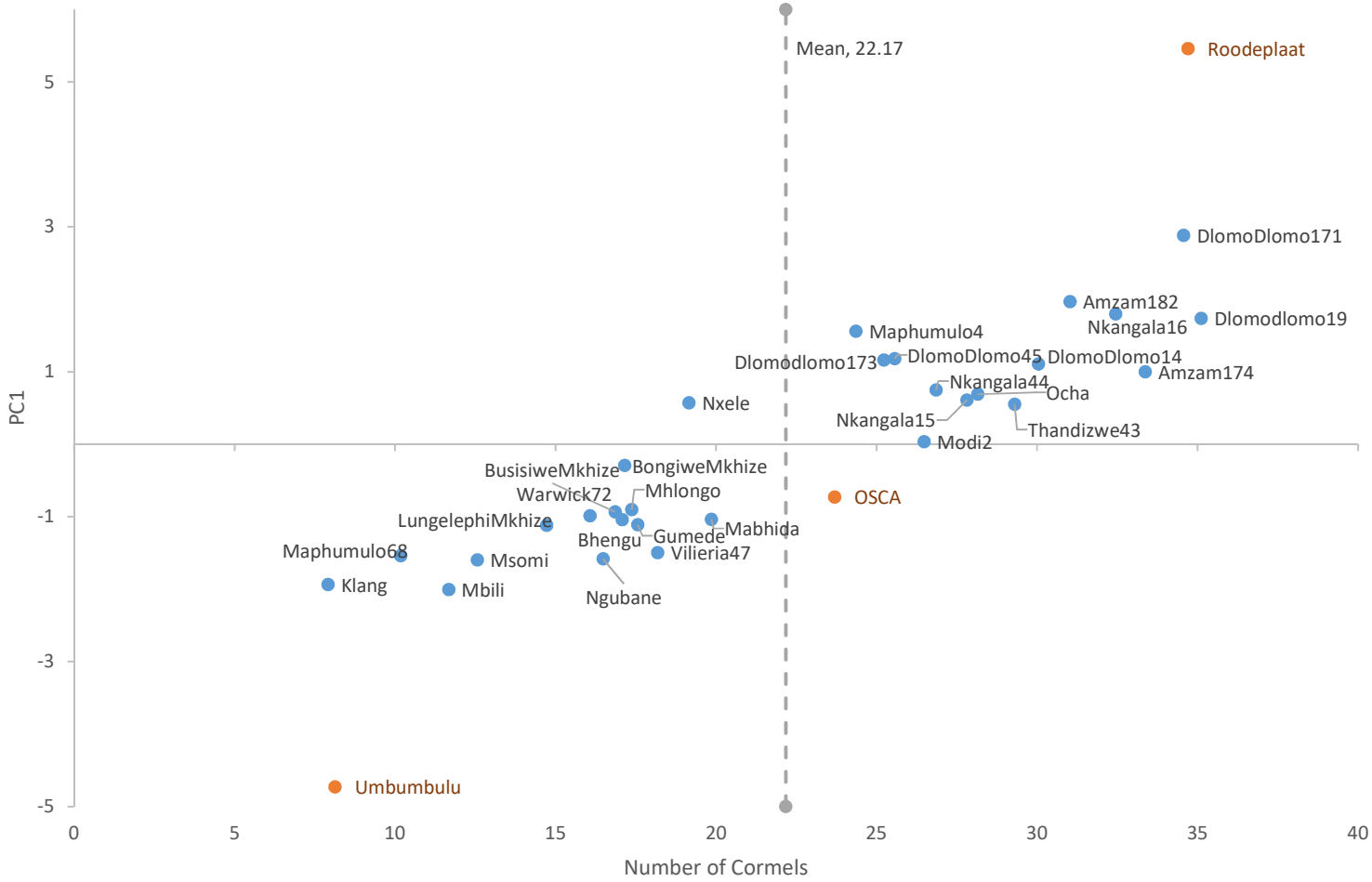

Figure 7: The AMMI1 model for number of cormels, plotting the overall mean of each line and locality against the first principal component (PC1).

8. Weight of cormels harvested from a single plant

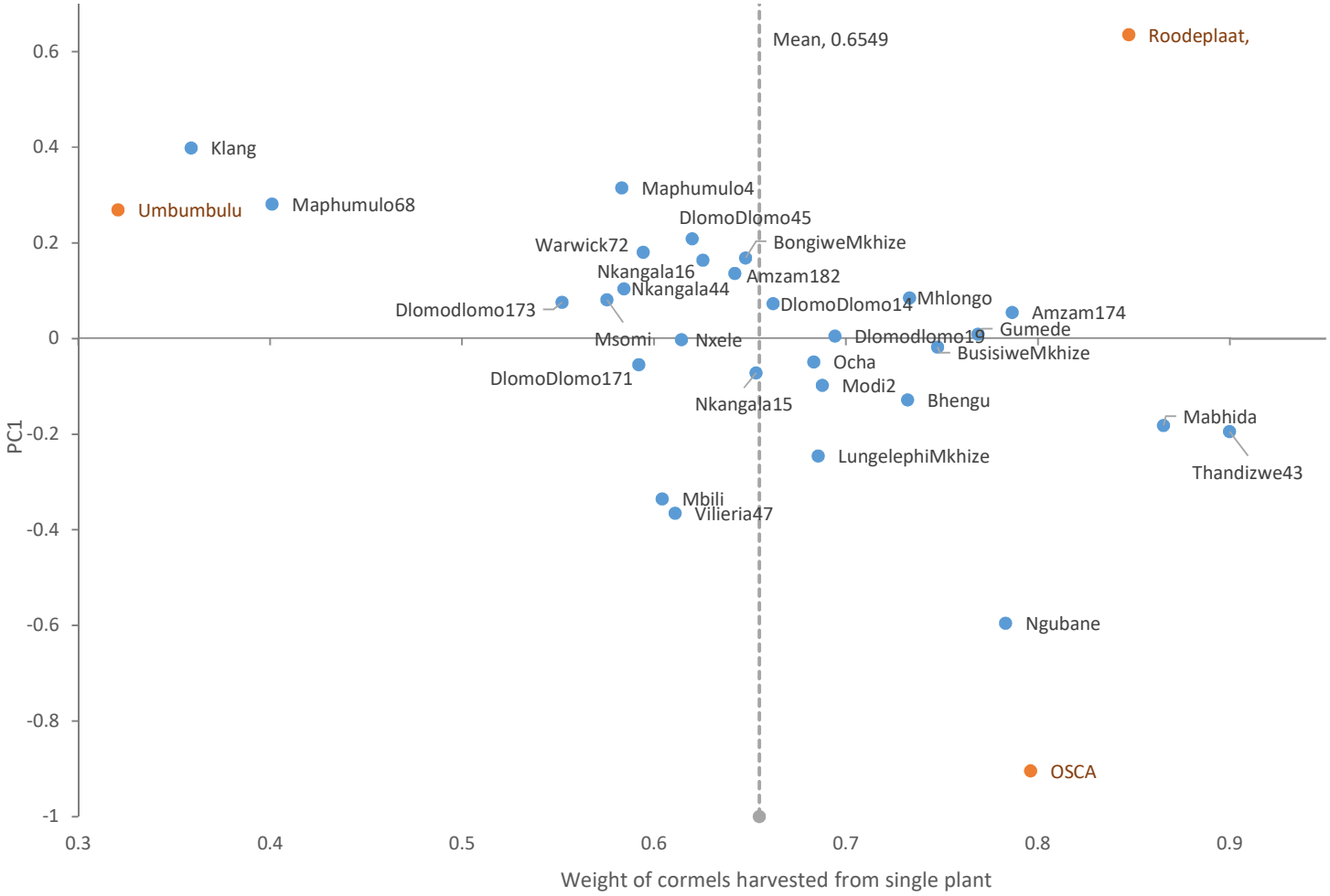

Table 8: The AMMI1 model for weight of cormels harvested from a single plant, plotting the overall mean of each line and locality against the first principal component (PC1).

9. Corm length

Figure 9: The AMMI1 model for corm length, plotting the overall mean of each line and locality against the first principal component (PC1).

10. Corm breadth

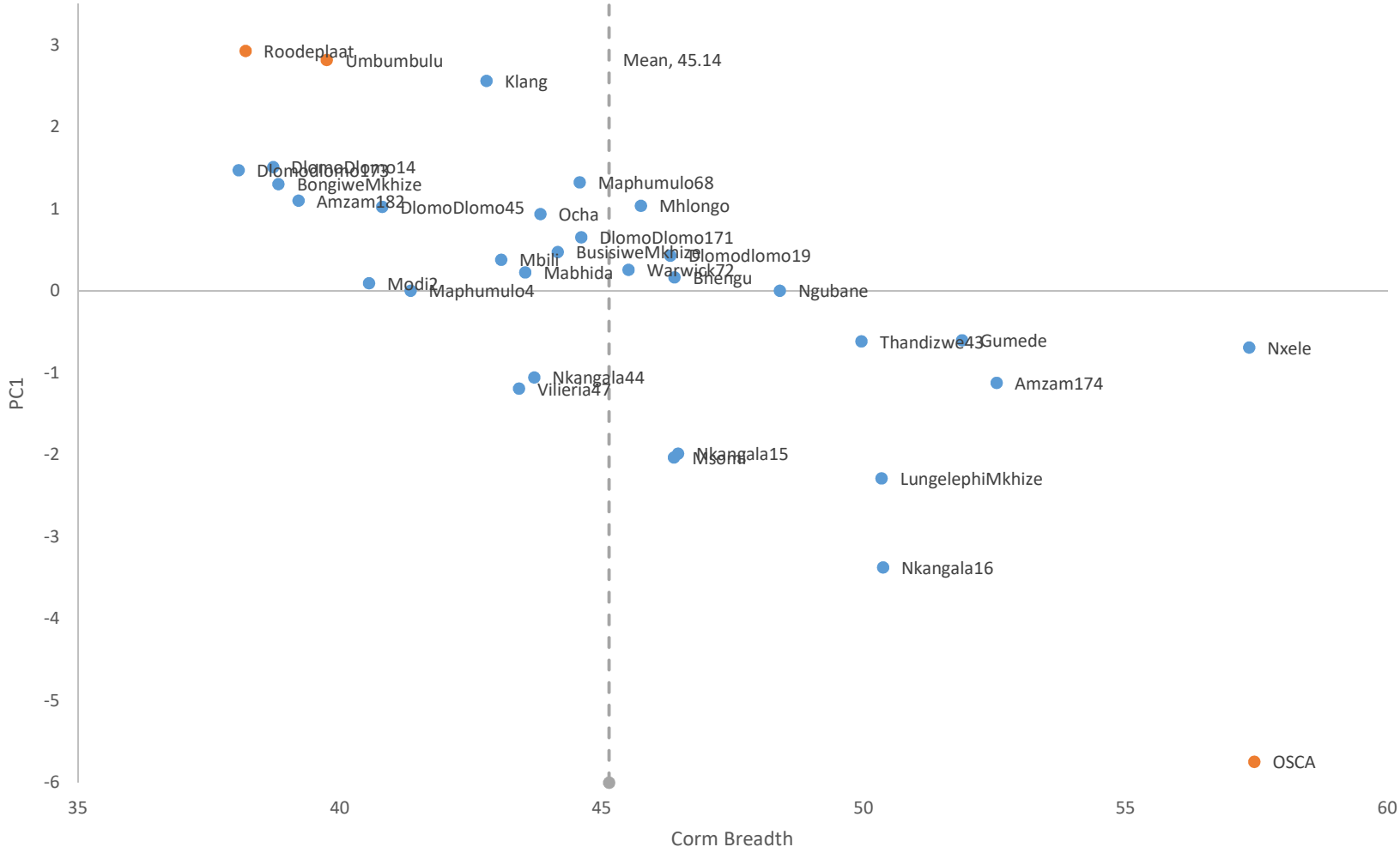

Figure 10 The AMMI1 model for corm breadth, plotting the overall mean of each line and locality against the first principal component (PC1).

Appendix 6: Summery of the genotypes performance

Table 1: Summary of the four top genotypes in the three different localities as well as overall taken from the ANOVA analysis

Characteristic	Locality	Top genotype	Second best genotype	Third best genotype	Fourth best genotype
Leaf Length	**OSCA**	Mbili	BongiweMkhize	DlomoDlomo171	Nkangala16
	Roodeplaat	Thandizwe43	Gumede	Ngubane	BongiweMkhize
	Umbumbulu	Vilieria47	Mbili	Ngubane	DlomoDlomo45
	Overall	**BongiweMkhize**	**Mbili**	**Ngubane**	**Thandizwe43**
Leaf Width	**OSCA**	Mbili	Amzam174	Vilieria47	Ngubane
	Roodeplaat	Gumede	Mabhida	Ngubane	BongiweMkhize
	Umbumbulu	Bhengu	Nkangala44	Modi2	Dlomodlomo19
	Overall	**Ngubane**	**Mbili**	**Mabhida**	**Amzam174**
Leaf number	**OSCA**	Thandizwe43	BusisiweMkhize	LungelephiMkhize	Ngubane
	Roodeplaat	Amzam174	Nkangala15	Bhengu	Amzam182
	Umbumbulu	Amzam174	Mabhida	Mbili	Klang
	Overall	**Amzam174**	**Thandizwe43**	**Nkangala15**	**DlomoDlomo45**
Plant Height	**OSCA**	BongiweMkhize	Ngubane	Nkangala16	Amzam174
	Roodeplaat	Ocha	Amzam182	Dlomodlomo19	Bhengu
	Umbumbulu	Amzam174	Dlomodlomo19	DlomoDlomo45	Modi2
	Overall	**Ngubane**	**Amzam174**	**Thandizwe43**	**BongiweMkhize**
Canopy diameter	**OSCA**	Amzam174	Thandizwe43	DlomoDlomo45	BongiweMkhize
	Roodeplaat	Amzam182	DlomoDlomo45	Thandizwe43	Amzam174
	Umbumbulu	Nkangala44	LungelephiMkhize	Mbili	Thandizwe43
	Overall	**Thandizwe43**	**Amzam174**	**Nkangala44**	**Amzam182**
Number of suckers	**OSCA**	Amzam174	DlomoDlomo171	DlomoDlomo45	Thandizwe43
	Roodeplaat	Mbili	Nkangala15	Amzam174	Thandizwe43
	Umbumbulu	Amzam174	Nkangala44	Amzam182	Thandizwe43
	Overall	**Amzam174**	**Nkangala15**	**Thandizwe43**	**DlomoDlomo171**

Characteristic	Locality	Top genotype	Second best genotype	Third best genotype	Fourth best genotype
Number of cormels	**OSCA**	Dlomodlomo19	Nkangala15	Thandizwe43	Amzam174
	Roodeplaat	DlomoDlomo171	Dlomodlomo19	Nkangala16	Amzam182
	Umbumbulu	Amzam174	Mbili	Ngubane	LungelephiMkhize
	Overall	**DlomoDlomo45**	**DlomoDlomo171**	**Amzam174**	**Nkangala16**
Weight of cormels	**OSCA**	Ngubane	Thandizwe43	Mabhida	Vilieria47
	Roodeplaat	Thandizwe43	Amzam174	BusisiweMkhize	Mhlongo
	Umbumbulu	Mabhida	Ocha	Amzam174	Gumede
	Overall	**Thandizwe43**	**Mabhida**	**Amzam174**	**Ngubane**
Corm length	**OSCA**	LungelephiMkhize	Amzam174	Nkangala16	Nxele
	Roodeplaat	BusisiweMkhize	BongiweMkhize	Klang	Nxele
	Umbumbulu	LungelephiMkhize	Ngubane	Maphumulo68	Bhengu
	Overall	**LungelephiMkhize**	**Amzam174**	**Nxele**	**Ngubane**
Corm breadth	**OSCA**	Nkangala16	LungelephiMkhize	Nxele	Amzam174
	Roodeplaat	Nxele	BusisiweMkhize	Mhlongo	Ocha
	Umbumbulu	Klang	Gumede	Amzam174	DlomoDlomo45
	Overall	**Nxele**	**Amzam174**	**Gumede**	**Nkangala16**

Table 2: Summary of the four top genotypes in the three different localities as well as overall taken from the AMMI analysis

Characteristic	Locality	Best Performer	Second Best Performer	Third Best Performer	Fourth Best Performer
Leaf Length	OSCA	Mbili	BongiweMkhize	DlomoDlomo171	Nkangala16
	Roodeplaat	Thandizwe43	Ngubane	BongiweMkhize	Gumede
	Umbumbulu	Thandizwe43	Gumede	DlomoDlomo45	Ngubane
	Overall	BongiweMkhize	Mbili	Ngubane	Thandizwe43
Leaf Width	OSCA	Mbili	Amzam174	Vilieria47	Ngubane
	Roodeplaat	Mabhida	Ngubane	Gumede	Bhengu
	Umbumbulu	Mabhida	Ngubane	Gumede	Bhengu
	Overall	Ngubane	Mbili	Mabhida	Amzam174
Leaf number	OSCA	Thandizwe43	BusisiweMkhize	LungelephiMkhize	Ngubane
	Roodeplaat	Amzam174	Thandizwe43	Nkangala15	Amzam182
	Umbumbulu	Amzam174	Amzam182	Bhengu	Nkangala15
	Overall	Amzam174	Thandizwe43	Nkangala15	Dlomodlomo19
Plant Height	OSCA	BongiweMkhize	Ngubane	Nkangala16	Amzam174
	Roodeplaat	Amzam174	Ngubane	Thandizwe43	Nkangala15
	Umbumbulu	Amzam174	Thandizwe43	Ngubane	DlomoDlomo14
	Overall	Ngubane	Amzam174	Thandizwe43	BongiweMkhize
Canopy diameter	OSCA	Amzam174	Thandizwe43	Dlomodlomo19	BongiweMkhize
	Roodeplaat	Thandizwe43	Nkangala44	Amzam174	Amzam182
	Umbumbulu	Nkangala44	Thandizwe43	Bhengu	Amzam182
	Overall	Thandizwe43	Amzam174	Nkangala44	Amzam182
Number of suckers	OSCA	Amzam174	Nkangala15	DlomoDlomo171	Thandizwe43
	Roodeplaat	Amzam174	Nkangala15	DlomoDlomo171	Thandizwe43
	Umbumbulu	Amzam174	Nkangala44	Amzam182	Thandizwe43
	Overall	Amzam174	Nkangala15	Thandizwe43	DlomoDlomo171
Number of cormels	OSCA	Dlomodlomo19	Amzam174	DlomoDlomo171	Nkangala16
	Roodeplaat	DlomoDlomo171	Dlomodlomo19	Nkangala16	Amzam182
	Umbumbulu	Amzam174	Dlomodlomo19	Thandizwe43	Modi2
	Overall	Dlomodlomo19	DlomoDlomo171	Amzam174	Nkangala16
Weight of cormels	OSCA	Ngubane	Thandizwe43	Mabhida	Vilieria47
	Roodeplaat	Amzam174	Mhlongo	Maphumulo4	Thandizwe43
	Umbumbulu	Thandizwe43	Mabhida	Amzam174	Gumede
	Overall	Thandizwe43	Mabhida	Amzam174	Ngubane

Characteristic	Locality	Best Performer	Second Best Performer	Third Best Performer	Fourth Best Performer
Corm length	OSCA	Amzam174	Nkangala16	LungelephiMkhize	Nxele
	Roodeplaat	LungelephiMkhize	Maphumulo68	Ngubane	Ocha
	Umbumbulu	Maphumulo68	LungelephiMkhize	Ngubane	Ocha
	Overall	LungelephiMkhize	Amzam174	Nxele	Thandizwe43
Corm breadth	OSCA	Nkangala16	LungelephiMkhize	Nxele	Amzam174
	Roodeplaat	Nxele	Klang	Gumede	Amzam174
	Umbumbulu	Nxele	Gumede	Klang	Amzam174
	Overall	Nxele	Amzam174	Gumede	Nkangala16

Table 3: Summary of the best genotypes according to the ANOVA and the AMMI analysis for each characteristic in each locality as well as the most stable in instable genotype for each characteristic.

Characteristic	Locality	ANOVA Best Performer	AMMI Best Performer	Stable	Instable
Leaf Length	OSCA	Mbili	Mbili		
	Roodeplaat	Thandizwe43	Thandizwe43		
	Umbumbulu	Vilieria47	Thandizwe43		
	Overall	BongiweMkhize	BongiweMkhize	Gumede	Klang
Leaf Width	OSCA	Mbili	Mbili		
	Roodeplaat	Gumede	Mabhida		
	Umbumbulu	Bhengu	Mabhida		
	Overall	Ngubane	Ngubane	Nkangala44	KLang
Leaf number	OSCA	Thandizwe43	Thandizwe43		
	Roodeplaat	Amzam174	Amzam174		
	Umbumbulu	Amzam174	Amzam174		
	Overall	Amzam174	Amzam174	DlomoDlomo171	LungelephiMkhize
Plant Height	OSCA	BongiweMkhize	BongiweMkhize		
	Roodeplaat	Ocha	Amzam174		
	Umbumbulu	Amzam174	Amzam174		
	Overall	Ngubane	Ngubane	Amzam182	Maphumulo4
Canopy diameter	OSCA	Amzam174	Amzam174		
	Roodeplaat	Amzam182	Thandizwe43		
	Umbumbulu	Nkangala44	Nkangala44		
	Overall	Thandizwe43	Thandizwe43	Mabhida	Maphumulo68
Number of suckers	OSCA	Amzam174	Amzam174		
	Roodeplaat	Mbili	Amzam174		
	Umbumbulu	Amzam174	Amzam174		
	Overall	Amzam174	Amzam174	Amzam182	DlomoDlomo171
Number of cormels	OSCA	Dlomodlomo19	Dlomodlomo19		
	Roodeplaat	DlomoDlomo171	DlomoDlomo171		
	Umbumbulu	Amzam174	Amzam174		
	Overall	DlomoDlomo45	Dlomodlomo19	Modi2	DlomoDlomo171
Weight of cormels	OSCA	Ngubane	Ngubane		
	Roodeplaat	Thandizwe43	Amzam174		
	Umbumbulu	Mabhida	Thandizwe43		
	Overall	Thandizwe43	Thandizwe43	Nxele	Ngubane
Corm length	OSCA	LungelephiMkhize	Amzam174		
	Roodeplaat	BusisiweMkhize	LungelephiMkhize		
	Umbumbulu	LungelephiMkhize	Maphumulo68		
	Overall	LungelephiMkhize	LungelephiMkhize	DlomoDlomo171	Nkangala16
Corm breadth	OSCA	Nkangala16	Nkangala16		
	Roodeplaat	Nxele	Nxele		
	Umbumbulu	Klang	Nxele		
	Overall	Nxele	Nxele	Ngubane	Nkangala16