GENETIC DIVERSITY ANALYSIS AND DETERMINATION OF CALCIUM OXALATE CRYSTALS IN SOUTH AFRICAN TARO (*COLOCASIA ESCULENTA)* ACCESSIONS.

by

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DECLARATION

This work has not been previously submitted for any degree at this university or any other university.

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DEDICATION

I would like to dedicate this work to my parents and three sisters, Kateule, Chanda and Mwenya who always saw the bigger picture in my little journey called life, their love and sacrifices are always unmatched.

ABSTRACT

Taro [*Colocasia esculenta* (L) Schott] belongs to the family Araceae. It is an important staple food crop grown mainly by small scale farmers in many parts of the world. Taro is also grown in South Africa from the costal parts of the northern Eastern Cape to the KwaZulu-Natal north coast. Although it is an important staple crop in South Africa, very little information exists on the genetic diversity of the crop. Knowledge of the genetic diversity of a crop is important for breeding programmes. The aim of this study is to assess the genetic diversity of taro using morphological and molecular techniques and to determine the calcium oxalate content of 25 South African taro accessions. This study showed that the aerial portions of taro are variable for most quantitative characters. Most of the morphological variation was due to lamina length, petiole length, lamina width and plant girth that explained 54% of the variance in principal component analysis. The number of raphides was able to divide the accessions into two groups, one with relatively low counts and the other with high counts. Ntumeni had the lowest raphide count of only 27 ±12 raphides and Modderfontein had the highest count with 1150 ±104 raphides. Twelve accessions having low raphide counts ranging from 27 ±12 to 147 ±28 raphides per cell have been identified. RAPD data separated the accessions into three main groups that were further divided into five subgroups. The accessions did not group according to geographical locations. The ITS2 sequence generated clustering patterns that were similar to that obtained from RAPDs. The variation in the ITS2 secondary structure of taro included one common motif that was present in all 25 accessions. Some motifs were only present in some accessions. The discovery of these motifs strengthens the potential of the ITS2 region as a taxonomic marker and a powerful barcode for taro. The ITS2 motifs provide the means of identifying each of the 25 accessions of taro. The high genetic diversity, morphological variation and accessions with low

calcium oxalate content found in this study provide taro breeders a selection of parent crops for the improvement of taro.

Keywords: *Colocasia esculenta*, RAPD, ITS2, secondary structure, genetic diversity.

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CHAPTER (1) ONE

1.1. Background

Colocasia esculenta (taro) is a vegetatively propagated root crop in the family Araceae (Kreike, Van Eck & Lebot 2004). The family contains about 100 genera and 1500 species that are mainly distributed in the tropical and subtropical regions of the world (Wang 1983). Taro is an important staple food crop for millions of people in developing countries in Asia, Africa and Central America (Sharma, Mishra & Misra 2008).

Taro is made up of three regions: the leaves, corm and roots. The peltate leaves grow from the apical bud at the top of the corm which is an underground plant stem that serves as a storage organ. The peltate leaf distinguishes taro from other edible aroids. The corms vary widely in size, color and number. The most important food source from the plant is the root (corms and cormels). Cormels are young corms produced by fully mature corms. However, the leaves, leaf stalks, and petioles are also used as a vegetable (Shange 2004). Runners are part of the stem that grow sideways just below the ground.

In South Africa, taro is known as amadumbe (idumbe = singular), which refers to the swollen underground stem (dumba = swell in Zulu). Taro is cultivated mainly in the subtropical coastal area starting at Bizana district in the Eastern Cape and includes the rest of coastal KwaZulu-Natal. There is less cultivation of the crop in the midlands and generally none in the northern parts of the province where the climate is drier and cooler (Shange 2004).

Ethno-archaeological records show that *Colocasia* originated from the lndo-Malayan region (Taylor, Hunter, Rao, Jackson, Sivan & Guarino 2010) and found its way into Africa through Madagascar around 300BC (Alexander & Coursey 1969). However, according to Gerstner

(1938), cited by Fox and Young (1982), taro was introduced to South Africa before the arrival of the Europeans.

Taro is rich in carbohydrates, proteins, minerals and vitamins. Taro is also used as a medicinal plant for the treatment of tuberculosis, ulcers, pulmonary congestion and fungal infections. In spite of the plants commercial value, few studies have been conducted to genetically improve the crop (Sharma et al. 2008).

The taxonomy and nomenclature of taro is not well established and cultivars are generally named from the place where they are collected. Taro exhibits a wide range of morphological variation both qualitatively and quantitatively (Trimanto, Sajidan & Sugiyarto 2010). The assessment of morphological characters of taro will be valuable in helping to taxonomically differentiate the accessions.

Taro contains calcium oxalate crystals that protect the plant from micro-organisms and herbivores (Bradbury & Nixon 1998). These are mainly found in the leaves and corms and give taro an unpleasant pungent taste (acridity). The acridity is caused by a protease that is attached to raphides. The raphides are sharp, arrow-like crystals of calcium oxalate that cause a severe burning sensation in the mouth and throat accompanied by swelling when taro is eaten raw (Oscarsson & Savage 2006). As a result taro must always be eaten cooked. Cooking improves digestibility, promotes palatability, improves keeping quality, and also makes root crops safer to eat (Onwueme 1999).

Although a wealth of genetic resources exist, attempts to conserve the germplasm of taro and use it to solve production problems has not been successful. An assessment of the genetic variation within taro is a prerequisite for initiating efficient breeding programmes for obtaining desirable genotypes (Kreike et al. 2004).

The earliest studies on genetic diversity in taro used cytological techniques to count chromosome numbers. Studies showed that diploids have 2n = 2x = 28 chromosomes; triploids are common with 2n = 3x = 42 chromosomes, while tetraploids (2n = 4x = 56) are extremely rare and may be weak-growing (Matthews 2004). Triploids are believed to have arisen when unreduced gametes (2x = 28) from one parent crossed with normal gametes from another parent (1n = 1x = 14). Early geographical surveys of chromosome numbers revealed that only diploids are common in the Pacific Islands, while diploids and triploids are common in the mainland of Asia (Lebot & Aradhya 1991; Sreekumari & Mathew 1991). Coates, Den and Gaffey (1988) examined chromosome morphology and found diverse cytotypes among diploids and triploids.

Insights into the genetic diversity among taro cultivars are useful in plant breeding and *ex situ* conservation of the plant's genetic resources. Taro germplasm characterization using molecular methods contributes to the knowledge of genetic relationships between accessions and therefore facilitates the breeding of taro cultivars to satisfy market needs and responses to diverse biotic (e.g., taro leaf blight) and abiotic (e.g., drought and salinity) challenges (Soltis, Soltis & Doyle 1992).

The purpose of this study is to: (i) assess the morphological diversity of taro, (ii) determine the calcium oxalate content by the quantification of raphides in the taro leaves, (iii) assess the genetic diversity using Random Amplified Polymorphic DNA (RAPD) markers, (iv) examine sequence variation of the Internal Transcribed (ITS) Spacer 2 regions, and (v) predict the ITS2 secondary structures of the taro accessions. This information may be useful in establishing breeding programmes for the enhancement of taro in South Africa.

1.2. Research aim

The aim of this study is to assess the genetic diversity and determine the calcium oxalate content of South African taro accessions.

1.3. Objectives of the study

1.3.1. To assess the morphological diversity of the aerial portions of 25 taro accessions.

1.3.2. To determine the calcium oxalate content of the taro accessions by quantification of raphides using cytological techniques**.**

1.3.3. To assess the genetic diversity of taro accessions using Random Amplified Polymorphic DNA (RAPD) markers.

1.3.4. To assess the sequence variation of the Internal Transcribed (ITS) Spacer 2 regions.

1.3.5. To predict the secondary structure models of the ITS2 sequences in taro.

CHAPTER (2) TWO

LITERATURE REVIEW

2.1. General introduction

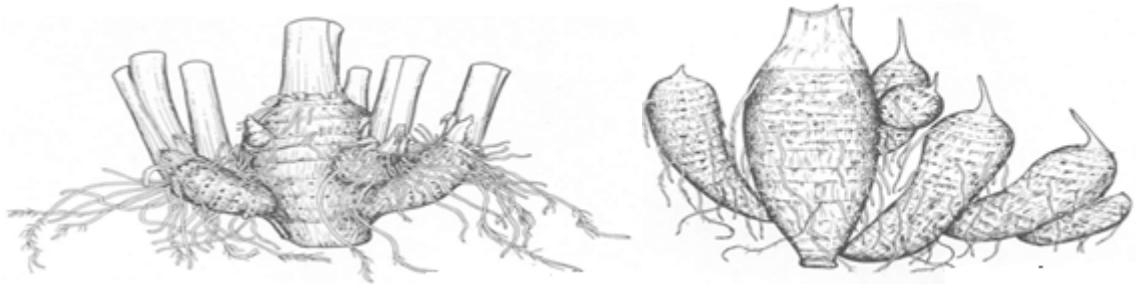
This chapter gives an overview on the taxonomy of taro, its morphological characters, and the importance of the food crop. It explains how it is used, its anti-nutritive properties (calcium oxalate crystals) and its production in South Africa and worldwide. Lastly it highlights the diversity studies conducted on the crop.

2.2. Taro taxonomy

Taro belongs to the genus Colocasia, within the sub-family Colocasiodeae of the monocotyledonous family Araceae. Due to its long history of vegetative propagation, there is considerable confusion in the taxonomy of the genus. Cultivated taro is classified as *Colocasia esculenta*, but the species is considered to be polymorphic. There are at least two botanical varieties (Purseglove 1972):

i) *Colocasia esculenta* (L.) Schott var. *esculenta*;

ii) *Colocasia esculenta* (L.) Schott var. *antiquorum* (Schott) which is synonymous with *C. esculenta* var. *globulifera* (Purseglove 1972).



(a) **(b)**

Fig. 1. Differences in corm structure between (a) *Colocasia esculenta var.esculenta,* and (b) *Colocasia esculenta var antiquorum*.

Colocasia esculenta var. *esculenta* produces a large cylindrical central corm, and very few cormels. It is referred to agronomically as the dasheen type of taro. *Colocasia esculenta* var. *antiquorum*, on the other hand, has a small globular central corm, with several relatively large cormels arising from it. This variety is referred to agronomically as the eddoe type.

2.3. Morphology characteristics of taro

2.3.1. Leaf

Taro has pale green or purple heart-shaped leaves (20 to 50 cm long) with long leaf stalks from 30 to 90 cm in length. The leaf is covered with a fine wax layer (Shange 2004). The lamina is oblong-ovate, glabrous, with rounded basal lobes. Three main veins radiate from the point of attachment of the petiole, one going to the apex, and one to each of the two basal lamina lobes. Some prominent veins arise from the three main veins, but the overall leaf venation is reticulate or net-veined (Onwueme 1999).

2.3.2. Petiole

The petiole is flared out at its base where it attaches to the corm, and it effectively clasps around the apex of the corm. The petiole is thickest at its base, and becomes thinner towards its attachment to the lamina. Internally, the petiole is spongy in texture with numerous air spaces which presumably facilitate gaseous exchange when the plant is grown in swampy or flooded conditions (Mare 2006).

2.3.3. Corms

Taro possesses enlarged, starchy, underground stems which are properly designated corms (Wang 1983). The shape varies from elongated to spherical with an average diameter of 15 to 18 cm. It is up to 30 cm long and constitutes the main edible part of the plant. The corms are highly variable with respect to hydration, size, color, and chemistry. The corm is composed outwardly

of concentric rings of leaf scars and scales. It bears one or more smaller secondary cormels which arise from lateral buds present under each scale or leaf base. Anatomically, the tuber is composed of a thick, brown outer covering (Wang 1983). In eddoe types, the corm is small, globoid, and surrounded by several cormels (stem tubers) and daughter corms. The cormels and the daughter corms together constitute a significant proportion of the edible harvest in eddoe taro. Daughter corms usually give rise to subsidiary shoots even while the main plant is still growing, but cormels tend to remain dormant and will only give rise to new shoots if left in the ground after the death of the main plant. Each cormel or daughter corm has a terminal bud at its tip, and axillary buds in the axils of the numerous scale leaves (Onwueme 1999).

2.4. Taro as a food crop

The nutritional value of a food depends upon its nutritional contents and their digestibility and the presence or absence of anti-nutrients and toxic factors. Taro is an important food staple in developing countries including Africa (Alcantara, Hurtada & Dizon 2013). Taro has a broad complement of vitamins and nutrients. The nutritional compositional of taro is given in (Table 1).

Table 1. Food composition of taro, components per 100g edible portion (Lambert 1982).

	Corms	Leaves	Petioles
Edible portion (%)	81	55	84
Energy (g)	85	69	19
Moisture (%)	77.5	79.6	93.8
Protein (g)	2.5	4.4	0.2
Fat (g)	0.4	1.8	0.2
Carbohydrate (g)	19	12.2	4.6
Fiber (mg)	0.4	3.4	0.6
Calcium (mg)	32	268	57
Phosphorus (mg)	64	78	23
Sodium (mg)	7	11	5
Potassium (mg)	514	1237	367
Iron (mg)	0.8	4.3	1.4
Vitamin (IU)	Trace	20385	335
Thiamine (mg)	0.1	0.1	0.01
Riboflavin (mg)	0.04	0.33	0.02
Niacin (mg)	0.9	2	0.2
Vitamin C (mg)	10	142	8

Taro contains about 7% protein on a dry weight basis. This is higher than that of yam, cassava or sweet potato (Onwueme 1999). Although the protein fraction is low in histidine, lysine, isoleucine, tryptophan, and methionine, it is rich in all the other essential amino acids. The protein content of the corm is higher towards the corm's periphery than towards its center. This implies that care should be taken when peeling the corm or else a disproportionate amount of the protein can be lost in the peel (Onwueme 1999). The taro leaf is also rich in protein containing

about 23% protein on a dry weight basis. It is also a rich source of calcium, phosphorus, iron, vitamin C, thiamine, riboflavin and niacin.

2.5. Different uses of taro

Taro is also used as a traditional medicine. The root extracts are used to treat rheumatism and acne, while leaf extracts are used for blood clotting at wound sites, neutralizing snake poison and as a purgative (Deo, Tyagi, Taylor, Becker & Harding 2009). Taro has become a symbol of cultural identification in different parts of the world (Deo et al. 2009).

Taro flour is used for infant formulas in the United States of America (Darkwa & Darkwa 2013). Its small starch grain and high digestibility (98.8%) makes it appropriate for food for infants, the elderly and the sick (Wang 1983). Products from taro are useful to individuals allergic to cereals and can be consumed by infants/children who are sensitive to milk (Darkwa & Darkwa 2013). Studies conducted in Asia have reported that babies who were fed 'poi'-a type of baby food prepared from taro were found to suffer less from diarrhea, pneumonia, enteritis and beriberi than babies fed with rice and bread (Wang 1983). Surveys of tooth decay conducted among Melanesian inhabitants of the Manus Islands showed that children who were fed on taro had better dental arches and protection from gum disease (Wang 1983). Taro can also be used as an additive to render plastics biodegradable (Kaushal, Kumar & Sharma 2013).

2.6. Anti-nutritive properties of taro

One of the factors that has impeded the full utilization of taro is that taro leaves, petioles, and corms contain an irritating or acrid agent (Wang 1983). Uncooked taro can cause a burning, itching sensation in the mouth and throat when eaten. It can cause similar sensations on the skin. The basic cause of the acridity is not well known. Some have believed it to be caused by calcium oxalate crystals that have bundles of needle-shaped crystals called raphides. Others suspected

that a chemical toxin is the cause of the acridity. Still others thought that it was due to mechanical irritation caused by the rubbing of sand like particles or druses which are immature forms of raphides in taro (Wang 1983). Druse crystal cells are found throughout the subepidermal mesophyll and rarely in the aerenchyma of taro leaves.

The existence of this acridity has made some form of cooking a prerequisite for the consumption of taro. Cooking can affect the soluble but not the insoluble oxalate content of the food. Boiling specifically at 80^{o}C reduces the soluble oxalate content of a food, if the cooking water is discarded; soaking, germination and fermentation will also reduce the content of soluble oxalates (Noonan & Savage 1999). In contrast, baking causes the oxalates to become more concentrated in the food due to the loss of water (Noonan & Savage 1999).

The bioavailability of the oxalates in food can be altered by the addition of extra calcium from milk and milk products (Oscarsson & Savage 2006). Brogren and Savage (2003) showed that when extra calcium was eaten together with spinach, an oxalate-rich food, the uptake of oxalates was reduced. Oscarsson and Savage (2006) suggested that taro leaves should be avoided by people who have an increased risk of calcium oxalate stone formation. Another option for at risk groups could be to consume cooked taro with milk as this decreases the amount of soluble oxalates that would be available for absorption in the small intestine (Oscarsson & Savage 2006). Taro leaves, particularly young leaves, will supply a significant amount of oxalate in the diet if they are eaten regularly.

2.7. Taro production in South Africa

Taro is grown in the subtropical coastal area of South Africa starting at Bizana district in the Eastern Cape and the rest of coastal KwaZulu-Natal. The crop is also cultivated in the subtropical and tropical parts of Mpumalanga and Limpopo provinces (Shange 2004).

Taro is grown by the Ezemvelo Farmers` Organization who market the product on contract to two large South African grocery store chains: Pick 'n' Pay and Woolworths (Modi 2003; Mare 2006). Marketing structures are in place for Umbumbulu subsistence farmers who are certified to supply taro to Woolworths (Mare 2006).

2.8. Production of taro worldwide

While taro is produced and consumed on a subsistence basis, quite a considerable amount is produced as a cash crop. Surpluses from the subsistence production manage to find their way to markets, thereby playing a role in poverty alleviation (Onwueme 1999). Worldwide, taro ranks fourteenth among staple vegetable crops with about 12 million tonnes produced globally from about 2 million hectares with an average yield of 6.5 t/ha (Rao, Hunter, Eyzaguirre & Matthews 2010). The (Table 2) shows the rankings of taro production from different countries. The highest production of taro was in Nigeria followed by China.

Table 2. United Nations Food and Agricultural Organization world ranking of taro by production in 2012

Rank	Country	Production (tonnes)
1	Nigeria	2,593,860
2	China	1,754,100
3	Cameroon	1,470,000
4	Ghana	1,354,800
5	Papua New Guinea	271,100
6	Madagascar	270,200
7	Rwanda	185,964
8	Japan	153,500
9	Egypt	119,379
10	Central African Republic	118,000
11	Philippines	110,761
12	Thailand	76,700
13	Cote d'Ivoire	70,000
14	Democratic Republic of the Congo	66,641
15	Fiji	60,283
16	Gabon	55,000
17	Solomon Islands	46,000
18	Sao Tome and Principe	27,500
19	Chad	25,700
20	Togo	20,165
21	Burundi	18,480
22	Samoa	17,500
23	Guinea	17,500
24	Dominica	17,100
25	Liberia	16,900
26	Comoros	10,600
27	American Samoa	10,000
28	Trinidad and Tobago	7,200
29	French Guiana	3,800
30	Tonga	3,600
31	Niue	3,100
32	Benin	2,300
33	Kiribati	1,900
34	Guyana	1,800

Production statistics, however, do not reflect the relative importance of taro in each of the countries. This is mainly because of distortion by land mass and population factors. China, for example, still manages to produce substantial quantities of taro which is a minor crop as compared to rice, because of the large land mass involved. A much better gauge of the importance of taro in each nation's food basket comes from examining the percentage of total dietary calories that each person derives from taro (Onwueme 1999).

Table 3 presents the top six countries (Tonga, Samoa, Solomon Island, Ghana, Gabon, and Papua New Guinea) in terms of the percentage of the dietary calories that is obtained from taro on a per capita basis.

Table 3. Percentage of dietary calories derived from taro and from all tubers in 1984 for various countries and continents.

	Taro %	All Tubers %
Tonga	18.1	45.0
Samoa	16.0	19.2
Solomon Island	7.7	39.0
Ghana	7.1	43.3
Gabon	4.6	36.7
Papua New Guinea	4.2	32.6
Congo DRC	0.1	56.8
Cameroon	0.5	44.5
Oceania	0.7	7.2
Asia	0.1	5.2
Africa	0.5	15.3
North and Central America	0.0	2.6
South America	0.0	6.4
Europe	0.0	4.7
World	0.1	6.0

2.9. Diversity studies in taro

The genetic diversity of cultivated forms of *C. esculenta* was initially analyzed using morphological and cytological characters (Kuruvilla & Singh 1981). The following in (Table 4) show some of the recent studies conducted on taro.

Table 4. Diversity studies conducted with molecular techniques in taro.

Technique used	Region	Accession number	Author and year
Karyotype	India	10	Das and Das (2014)
DNA microsatellite	Kenya,Tanzania,Uganda	98	Macharia (2013)
Chloroplast genome	New Zealand, Australia Pakistan, Japan	10	Ahmed et al. (2013)
SRAP	China	65	Liang et al. (2012)
RAPD and ISSR	Andaman Islands	21	Singh et al. (2012)

Das and Das (2014) looked at the genetic diversity of ten drought resistant cultivars of Indian taro. This involved the karyotype and genome size analysis of taro. Karyotype analysis revealed genotype specific chromosomal characteristics and structural alterations in the chromosomes of the genome, with variations of ploidy from 2n = 2 × = 28 (cv. Mothan, cv. Muktakeshi, cv. Sree Kiran, cv. Sree Pallavi, cv. Sunajhili) to 3n = 3 × = 42 (cv. Banky, cv. DP-25, cv. Duradin, cv. H-3, cv. Telia).

Macharia (2013) established the genetic diversity of taro in East Africa using DNA microsatellite markers. Plant material consisted of 98 taro cultivars obtained from East Africa (Kenya, Tanzania and Uganda). Six micro-satellite primers previously shown to reveal high level of polymorphism in Polynesian taro were used to analyze the samples. Population diversity estimates was high for accessions obtained from the Lake Victoria basin and Analysis of

molecular variance (AMOVA) revealed that most of the variation among individuals occurred within this population. Principal component analysis of SSR data indicated variation but did not show any geographical structure.

The chloroplast genome of taro revealed levels of genetic variation suitable for high-resolution phylogeographic and evolutionary studies of taro (Ahmed, Matthews, Biggs, Naeem, McLenachan and Lockhart, 2013). Liang, Liu, Liu, Fang, Yang and Li (2012) looked at the genetic diversity and relationship of 65 taro accessions using Sequence-related Amplified Polymorphism (SRAP) markers. The accessions were separated into 5 main groups based on the UPGMA. This classification was consistent with some morphological characteristics.

Singh, Singh, Faseela, Kumar, Damodaran and Srivastava (2012) found high genetic diversity of taro accessions growing naturally in Andaman Islands using RAPD and ISSR markers. The 21 accessions were grouped into two major clusters with both RAPD and ISSR markers with 56 and 57% diversity, respectively.

2.9.1. ITS region and RNA secondary structures

Eukaryotic nuclear ribosomal DNA (rDNA) has two internal transcribed spacers. The first (ITS-1) is located between the small subunit (16S-18S) and 5.8S rRNA cistronic regions, and the second (ITS-2) is located between the 5.8S and large subunit (23S-28S) rRNA cistronic regions.

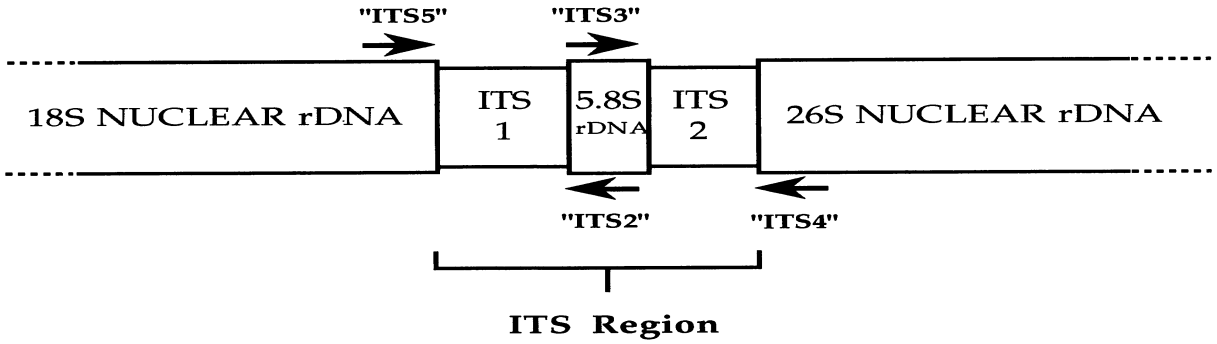


Fig. 2. Organization of the ITS region: arrows indicating the orientation and approximate position of primer sites.

The two spacers and the 5.8S subunit are collectively known as the internal transcribed spacer (ITS) region and have become an important nuclear locus for molecular systematic investigations of flowering plants (Baldwin, Sanderson, Porter, Wojciechowski, Campbell & Donoghue 1995). The popularity of the ITS region can be attributed to the relatively high rate of nucleotide substitution in the transcribed spacers, permitting the systematic comparison of relatively recently diverged taxa. In addition, the ITS region can be readily PCR-amplified and sequenced with conserved primers positioned in the cistronic regions (Liston, William, Robinson & Oliphant 1996). Defining a single general marker for the taxonomic classification of an organism on all taxonomic levels is challenging. On the one hand, one needs a fast-evolving marker for classification on the species level; on the other hand this marker will be too divergent for reconstructing the phylogeny on a higher level. This problem is usually addressed by using different markers like the ITS for low level and 18S or 28S rDNA for high level classification. Recently, it was suggested, that the internal transcribed spacer 2 region (ITS2) of the nuclear rDNA cistron might be a marker suitable for taxonomic classification over a wide range of levels (Coleman 2003). The ITS2 intergenic sequence is located between the 5.8S and 28S ribosomal genes. It can evolve 30 times slower (Caccone, Gentile, Burns, Sezzi, Bergman, Ruelle, Saltonstall & Powell 2004) than mitochondrial DNA (mtDNA) and has been shown to be an important part of ribogenesis (Joseph, Krauskopf, Vera & Michot 1999). Although the ITS is not incorporated into the mature rRNA, they encode signals for proper processing of the rRNA transcripts, which itself depends on the secondary structure of ITS RNA (Hillis & Dixon 1991). In contrast to the ITS1 spacer, secondary structures of functional ITS2 spacer as well as 5.8S rDNA sequences are highly conserved within plants (Hershkovitz & Zimmer 1996). The ITS2 spacer forms a four helices structure with typical pyrimidine-pyrimidine bulge in helix II and the

most conserved primary sequence includes the 5'-UGGU-3' in the helix III (Coleman 2007). The 5.8 S rDNA sequences contain three conserved motifs in their nucleotide sequences that are essential for the correct folding of secondary structures (Harpke & Peterson 2008a). The conserved secondary structure of ITS2 and 5.8S sequences makes it possible to identify divergent ITS paralogs with non-functional pseudogenes (Harpke & Peterson 2008b). Despite variability in the ITS2 sequence and length, its rRNA secondary transcript structure contains a highly conserved core (Coleman 2003). This highly conserved core is common to all eukaryotes and should be useful for evaluating the relationships of closely related organisms (Coleman 2007).

CHAPTER (3) THREE

METHODOLOGY

3.1. Sample collection and storage

Plant material was obtained from the germplasm bank at the Agricultural Research Council-Vegetable and Ornamental Plant Institute (ARC-VOPI) in Roodeplaat. The institute originally collected the plant material from the locations shown in (Fig. 3). The plants were regrown in the greenhouse (Fig. 4) at the Vaal University of Technology for morphological character assessment, cytological analysis and DNA extraction.

Fig. 3. A map of South Africa showing the locations (tagged with yellow pins) from where the 25 accessions of taro were collected.

Fig. 4. Taro accessions grown in the greenhouse at the Vaal University of Technology.

3.2. Morphological character analysis

Morphological character analysis was carried out when the plants reached maturity at 170 days after planting. The following parameters were measured to assess the morphological diversity among the taro accessions:

(i) Petiole length: The length between the base of the plant petiole in contact with the soil and point of attachment with the leaf (in cm).

(ii) Lamina length: The length of the lamina was measured from the leaf base to the leaf apex (in cm), along the longest side of the leaf.

(iii) Leaf breadth: It was the measurement of the leaf breadth from one end of the leaf margin to the other end across the leaf lamina (in cm).

(iv) Leaf number: Total number of leaves per plant.

(v) Plant girth: Measurements of the circumference of the stem 2cm from the soil surface.

(vi) Leaf colour

(vii) Petiole colour.

(viii) Lamina vein count.

3.3. Calcium oxalate quantification

Calcium oxalate quantification was achieved by using the method described by Sunell and Healey (1985). Young fully unfurled leaves were chosen randomly from each plant. The entire leaf was cleared by first placing them in 70% ethanol at 60^{o}C for 6 hr and then in 95% ethanol at room temperature for 1 hr. After a brief wash in distilled water, the leaves were placed in 5% NaOH for 1 hr at room temperature, and then washed three times in distilled water to completely remove chlorophyll and clear the leaf. Transections 2cm wide were cut from three regions: the leaf-base, middle lamina and mid rib. The transections were then mounted adaxial side up in glycerol on glass slides and then viewed under the microscope (Olympus CX31 Bright field microscope at X40 magnification). The number of cells containing raphides (calcium oxalate crystals) was determined by counting small areas (0.252 sq mm) along all leaf transects using 20 fields per leaf section from the left margin to the right margin. Each plant was sampled three times for standard statistical purposes.

3.4. DNA extraction

DNA was extracted from young leaves according to the CTAB method (Saghai – Maroof, Soliman, Jorgensen & Allard 1984; Doyle & Doyle 1987). Five grams of young leaves was ground in liquid nitrogen to a fine powder with a mortar and pestle and then 15 ml hexadecyltrimethylammonium bromide (CTAB) isolation buffer (1M Tris pH 8.0, 0.5M Ethylenediaminetetraacetic acid Di-sodium salt, 5M NaCl, distilled water, 20g polyvinyl pyrrolidone 40, 20ml mercaptoethanol) added and mixed vigorously. The suspension was incubated at 65^{o}C for 30 min and then allowed to cool for 2-5 min. A volume of 10 ml chloroform/isomyl alcohol (24: 1 v/v) was added to the suspension and mixed gently. The suspension was centrifuged at 6,000 rpm for 10 min and the supernatant extracted with a

micropipette into a fresh oak ridge tube. The DNA was precipitated by adding 2/3 volume of ice cold isopropanol to the supernatant, mixed gently and the contents centrifuged at 6,000 rpm for 10 min. The pellet was rinsed with 70% ethanol and allowed to dry at room temperature. The pellet was then resuspended in 200 ul Ethylenediamine Tetra acetic Acid; buffered solution, (TE buffer) and left at room temperature to dissolve fully. The RNA was digested by adding 10 µl RNase (10 mg/ml) and incubated at 37^{o}C for 30 min. A volume of 1/10 3 M sodium acetate was added followed by 2 volumes of cold 99% ethanol and the mixture was centrifuged at 6,000 rpm for 5 min. The pellet was then washed with 70% ethanol and allowed to dry briefly at room temperature. The DNA samples were quantified using the Nano drop 2000C spectrophotometer (Thermo Scientific, Wilmington, Delaware) and standardized to 40 ng/µl using TE buffer.

3.5. RAPD analysis

Reaction mixtures for RAPD analysis consisted of 3 µl DNA, 1.5 µl 37.5 mM $MgCl_2$, 3.0 µl 2.5 mM each dNTP, 2.0 µl primers (Operon Technologies, Alameda, CA) , 3.88 µl nuclease free water, 1.5 10x amplification buffer, 0.12 µl *Taq* polymerase (Thermo Scientific, Foster, CA) in a total volume of 15 µl. Amplifications were carried out in a Bioer XP cycler (Bioer Technology Co., Ltd, Hangzhou, China) with the following amplification conditions: an initial 3 min denaturation at 94^{o}C followed by 35 cycles of 50s at 94^{o}C, 50s at 40^{o}C, and 1.5 min at 72^{o}C, with a final extension step of 7min at 72^{o}C. Approximately 15 µl of the Polymerase Chain Reaction (PCR) product was separated on a 1% agarose gel containing ethidium bromide in 1 x TBE buffer. A 1 kilo base pair Kapa Biosystems ™ universal molecular weight marker (Kapa Biosystems ™, Wilmington, MA) molecular weight marker was used. The gel was then observed under the Bio-Rad Molecular Imager Gel Doc XR™+ System using UV light, (Bio-Rad, Hercules, CA).

3.6. PCR amplification and sequencing of the ITS2 region

Components for 25 µl PCR reactions were: 3 µl of DNA, 3 µl of ITS3 (GCATCGATGAAGAACGCAGC), and ITS4 (TCCTCCGCTTATTGATATGC), 2 µl $MgCl_2$, 9.8 µl distilled water, 2µl amplification buffer, 0.1 mM of each dNTP (dGTP, dCTP, dATP and dTTP), and 0.2 µl GoTaq® polymerase (Promega, Madison, WI). The amplification conditions for PCR were as follows: 40 cycles, each cycle consisting of a denaturation step at 94 °C for 1 min, an annealing step at 50 °C for 1 min and an extension step at 72 °C for 1 min 30 s. After the 40th cycle, a final extension step was performed at 72°C for 7 min. The amplification reactions were performed in a Bio-Rad C1000 Thermo Cycler™ (Bio-Rad, Hercules, CA). After amplification, the products were separated by electrophoresis on a 1 % agarose gel immersed in TBE buffer to determine the size of the amplicons (90 mM Tris-borate, 2 mM EDTA, pH 8.0). Following amplification, the products were sequenced at Inqaba Biotech (Pretoria, South Africa).

3.7. Data analysis.

3.7.1. Morphological character analysis

Statistical Package for Social Sciences (SPSS) was used for the Principle Component Analysis (PCA) of the morphological characters. PCA indicated the characters on the basis of the highest factor loading as a reduction method. It was performed to determine the correlation between characters and was used to find the most significant traits contributing to variation through the generation of eigen values.

3.7.2. Calcium oxalate analysis

Calcium oxalate crystals were quantified from three different parts of each leaf. This was repeated from different leaves atleast 3 times. The averages and mean standard errors of the counts were recorded. A One-way Analysis of Variance (ANOVA) was carried out to determine

whether there were any significant differences between the mean counts of the raphides containing crystals.

3.7.3. Analysis of RAPD data

The RAPD patterns for each primer were scored as band presence (1) and absence (0). Data was then entered into a data base programme (Microsoft Excel) and compiled into a binary matrix for phenetic analysis using Numerical Taxonomy and Multivariate Analysis System (NTSYS). A dendrogram was derived using unweighted pairwise group method with arithmetical average (UPGMA) basing the findings on Jaccard's similarity coefficient.

3.7.4. ITS2 sequences analysis

The sequence chromatograms were visualized and edited using Chromas Lite 2.1.1 (Technelysium Pty, Brisbane, Australia). Alignments from these sequences were then made using ClustalW (Bioinformatics Center, Institute for Chemical Research, Kyoto University, Kyoto Japan). All sequences were aligned and appropriate cut-offs were applied.

3.7.5. ITS2 dendrogram analysis

The taxonomic relationships among the 25 taro sequences were determined using the Molecular Evolutionary Genetics Analysis version 5 software (MEGA5) (http:// www.megasoftware.net/). The dendrogram was generated from the ITS2 sequence data, generated using the neighbour-joining method (Saitou & Nei 1987).

3.7.6. Secondary structure prediction using ITS2 sequences

Secondary structures were derived from the 25 taro ITS2 sequences by using the Sfold web server. Sfold predicts secondary structures based on statistical sampling paradigm to fold nucleic acids (http://sfold.wadsworth.org/cgi-bin/sirna.pl), (Ding & Lawrence 2003). Default settings were used to derive the models.

CHAPTER (4) FOUR

RESULTS

4.1. Introduction

This chapter describes the results obtained for the morphological characterization of the taro accessions, calcium oxalate crystal quantification and genetic diversity with RAPD, ITS2 amplification, sequencing and secondary structure prediction.

Morphological characters of the aerial potions are shown in (Table 5). The 25 accessions were assessed on six quantitative characters and two qualitative characters.

Table 5. Morphological characters of the aerial portions of 25 taro accessions.

Name	Lamina width(cm)	Lamina length(cm)	Petiole length(cm)	Plant girth (cm)	Number of Lamina Veins	Leaf Color	Petiole colour	Total number of leaves
Makatini Res	5	7	18	5	24	Green	Green	2
Nelspruit(Vanuatu)	7	12	7	3	24	Purple	Green	2
Nigeria	23	30	41	7	16	Green	Purple	3
Makatini	14	20	44	5	18	Green	Green	2
Durban	10	18	40	5	18	Green	Purple	3
Tshwane 1	7	13	25	4	14	Green	Green	3
Ntumeni	8	12	20	4	12	Green	Green	2
Modderfontein	30	20	25	9	14	Green	Green	4
Empangeni	9	13	24	3	14	Green	Green	4
Lusikisiki	10	14	18	4	12	Green	Green	3
Hluhluwe	11	16	24	6	16	Green	Green	4
Umbumbulu	8	12	25	4	16	Green	Green	3
Tshwane 2	13	19	36	5	16	Green	Green	2
Cocoindia	11	17	29	4	16	Green	Green	3
Eshowe 1	11	14	24	4	16	Green	Green	3
Pietermaritzburg	9	13	23	3	16	Green	Green	2
Willowvale	12	17	33	3	14	Green	Green	2
Maphumulo	9	14	24	3	14	Green	Green	2
Isipingo	12	17	36	4	16	Green	Green	2
Mangozi	7	10	22	4	16	Green	Green	3
Eshowe 2	9	13	28	3	18	Green	Green	2
Ghana	6	8	19	2	16	Green	Green	2
Jozini	4	5	12	2	16	Green	Green	2
Mbazwana	10	12	28	3	16	Green	Green	2
Tshwane 3	7	9	20	4	16	Green	Green	3

4.2. Quantitative characters (Phenotypic Correlation)

The (Table 6) shows an inter-item correlation matrix of the morphological characters in SPSS extracted for principle component analysis.

Table 6. Inter-item correlation matrix for the aerial characters of taro.

	Lamina width	Lamina length	Petiole length	Plant girth	Lamina veins	Total leaves
Lamina Width	1.00					
Lamina length	.80	1.00				
Petiole length	.47	.70	1.00			
Plant girth	.83	.71	.36	1.00		
Lamina veins	-.23	-.18	-.01	-.26	1.00	
Total leaves	.39	.25	.09	.48	-.30	1.00

The (Table 7) indicates how much of the variability occurs in the dataset. The ideal variances would be 100% but usually analyses are within 60%-70% variance, with eigen values which are the actual variances of the principle component.

Table 7. Total variance of components with eigen values and sums of squared loadings.

Component	Initial Eigen values		Extraction Sums of Squared Loadings		Rotation Sums of Squared Loadings	
	%Variance	%Cumulative	%Variance	%Cumulative	%Variance	%Cumulative
1	53.8	53.8	53.8	53.8	46.5	46.5
2	20.2	73.9	20.2	73.9	27.4	73.9
3	12.2	86.1				
4	8.9	95.0				
5	2.8	97.8				
6	2.2	100.0				

The Kaiser-Meyer-Olkin Measure of Sampling Adequacy and Bartlett's Test of Sphericity are tests that provide a minimum standard which should be passed before principle component analysis is conducted (Table 8). Kaiser-Meyer-Olkin Measure of Sampling Adequacy measures between 0 and 1, and values which are closer to 1 considered better with a value of 0.6 being minimum. Bartlett's Test of Sphericity tests the null hypothesis that the correlation matrix is an identical matrix.

Table 8. Kaiser-Meyer-Olkin index and Bartlett's Test of Sphericity.

Kaiser-Meyer-Olkin Measure of Sampling Adequacy.		.742
Bartlett's Test of Sphericity	Approx. Chi-Square	71.893
	Df	15
	Sig.	.000

The (Table 9) shows component loadings which are correlations between the variables (plant characters) and the components. These are correlation values that range between -1 and 1.The columns under the headings are the principle components that have been extracted.

Table 9. Pattern matrix showing morphological characters with component loadings.

Characters	Component	
	1	2
Lamina length	.938	
Petiole length	.859	.314
Lamina width	.808	
Plant girth	.693	-.424
lamina veins		.767
Total leaves		-.754

4.3. Calcium oxalate crystal counts

The number of raphides containing calcium oxalate crystals in the 25 taro accessions is shown in (Table 10). The number was obtained from an average of three counts from three different parts of the leaf. The mean and standard error of the means are provided.

Table 10. The number of raphides containing calcium oxalate crystals in each of the 25 taro accessions.

	Count 1	Count 2	Count 3	Sum	Mean	±SEM
Ntumeni	16	50	15	81	27	11.50
Makatini	22	50	25	97	32.33	8.88
Empangeni	16	30	70	116	38.67	16.18
Cocoindia	1	90	30	121	40.33	26.20
Nelspruit(Vanuatu)	35	45	60	140	46.67	7.26
Eshowe 1	50	60	160	270	90.00	35.12
Tshwane 3	50	170	50	270	90.00	40.00
Makatini Res	88	105	90	283	94.33	5.36
Isipingo	63	100	144	307	102.33	23.41
Hluhluwe	50	150	175	375	125.00	38.19
Jozini	167	100	120	387	129.00	19.86
Tshwane 2	90	180	170	440	146.67	28.48
Mangozi	150	170	220	540	180.00	20.82
Durban	170	250	240	660	220.00	25.17
Tshwane1	200	165	310	675	225.00	43.68
Ghana	250	340	270	860	286.67	27.28
Mbazwana	185	470	220	875	291.67	89.74
Pieternaritzburg	170	380	400	950	316.67	73.56
Eshowe 2	260	450	360	1070	356.67	54.87
Lusikisiki	360	380	390	1130	376.67	8.82
Nigeria	574	730	450	1754	584.67	81.00
Maphumulo	100	800	1100	2000	666.67	296.27
Willowvale	860	720	780	2360	786.67	40.55
Umbumbulu	1100	800	900	2800	933.33	88.19
Modderfontein	1200	1300	950	3450	1150.00	104.08

The ANOVA results (Table 11) showing the source of variation, between the groups, within the groups and the significance of the test (P-value).

Table 11. Results of ANOVA for the variation in raphide counts.

ANOVA						
Source of Variation	SS	Df	MS	F	P-value	F crit
Between Groups	6629800	24	276241.7	16.19383	0.000	1.73708
Within Groups	852922.7	50	17058.45			
Total	7482723	74				

4.4. Molecular diversity results

4.4.1. DNA extraction

DNA was extracted from all 25 taro accessions as described in 3.4 of the methodology. All DNA extraction procedures resulted in high molecular weight DNA at high concentrations (Fig.5) and were sufficient for all reactions. The (Table 12) indicates the sample names corresponding to lanes 1-25 shown on the agarose gels.

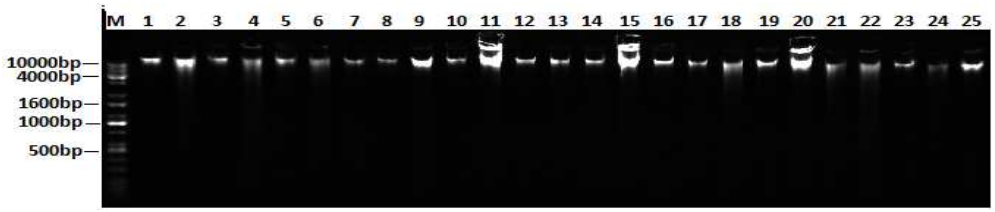


Fig .5. Agarose gel electrophoresis showing molecular DNA extracted from 25 taro accessions (bp): base pairs, (M): Kapa Bio systems™ universal molecular weight marker, lanes numbered 1-25.

Table 12. The sample names corresponding to the lane numbers 1-25 on the agarose gels.

Sample name	Lane
Hluhluwe	1
Maphumulo	2
Mbazwana	3
Willowvale	4
Lusikisiki	5
Isipingo	6
Pietermaritzburg	7
Eshowe 1	8
Eshowe 2	9
Empangeni	10
Tshwane1	11
Tshwane2	12
Tshwane3	13
Umbumbulu	14
Mangozi	15
Cocoindia	16
Ghana	17
Jozini	18
Durban	19
Makatni res	20
Makatni	21
Modderfontein	22
Nigeria	23
Ntumeni	24
Nelspruit	25

Following DNA extraction, the taro samples were subjected to RAPD analysis using 20 sets of primers. An example of the banding patterns produced from the primer OPB 03 is given in (Fig.6).

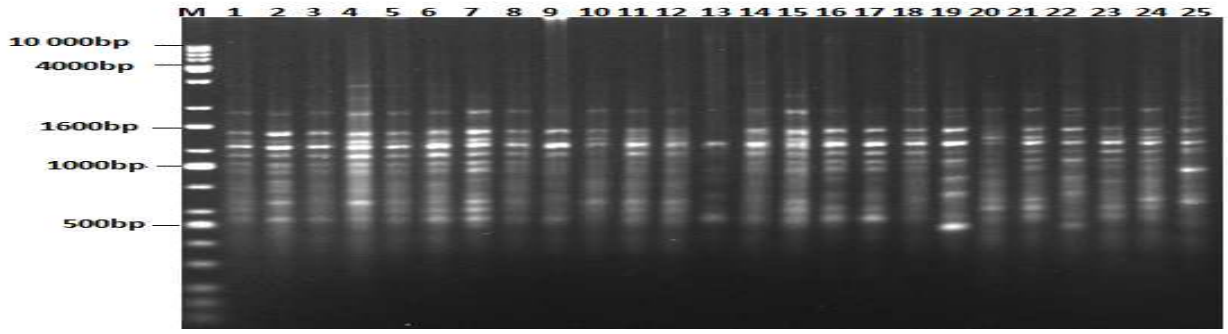


Fig. 6. Banding patterns produced by RAPD Primer OPB 03. (bp): base pairs, (M): Kapa Biosystems™ universal molecular weight marker, lanes numbered 1-25.

4.4.2. RAPD polymorphism

The (Table 13) shows the calculation of polymorphism in percentage form from the total number of bands produced from the amplified PCR products with the respective primers.

Table 13. Primers, primer sequences, total number of amplified products, number of polymorphic amplification products and percent polymorphism.

Primer	Sequence 5'-3'	Total No of amplified products	No of polymorphic amplification products	Percent(%) polymorphism
OPB 2	TGATCCCTGG	129	67	52
OPB 5	TGCGCCCTTC	159	95	60
OPD 5	TGAGCGGACA	86	64	74
OPC 7	GTCCCGACGA	116	59	51
OPB14	TCCGCTCTGG	166	83	50
OPD 14	TCTGGTGAGG	158	92	58
OPD 16	AGGGCGTAAG	140	90	64
OPD 17	TTTCCCACGG	117	83	71
OPB 20	GGACCCTTAC	87	86	98
Total		1158	719	62

The average number of bands per primer was 6. The maximum numbers of bands were produced with OPB 05, OPB 14, OPD 14 and OPD 17. The extent of polymorphism was observed to be high with five out of the nine primers: OPB 20, OPD 5, OPD 17, OPB 5 and OPD 16 with 60-98% polymorphism.

4.4.3. Dendrogram generated from RAPD

The binary matrix was obtained by scoring the presence and absence of amplification products. This was then subjected to UPGMA basing the findings on Jaccard's similarity coefficient. The dendrogram obtained from the UPGMA analysis grouped 25 accessions into three clusters I, II and III then subdivided further into sub clusters Ia, Ib, Ic and cluster IIa and IIb respectively, from the RAPD dendrogram (Fig. 7).

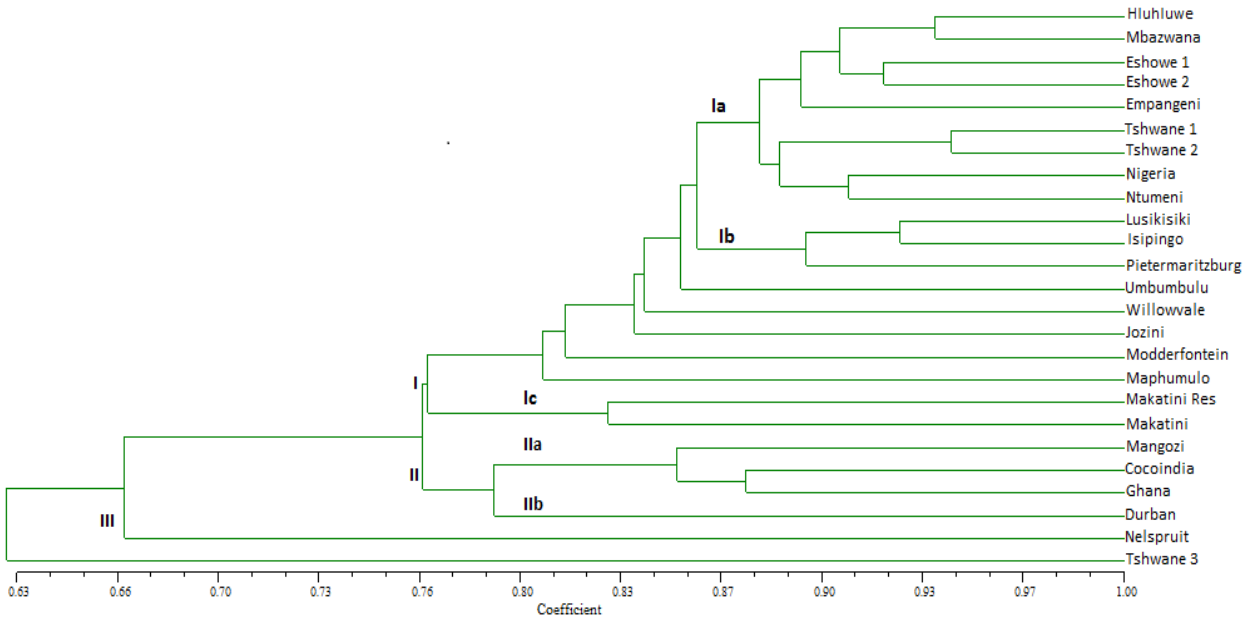


Fig.7. Dendrogram derived from RAPD data .

The frequency distribution of Jaccard's similarity coefficient values for RAPD are given in (Fig. 8).

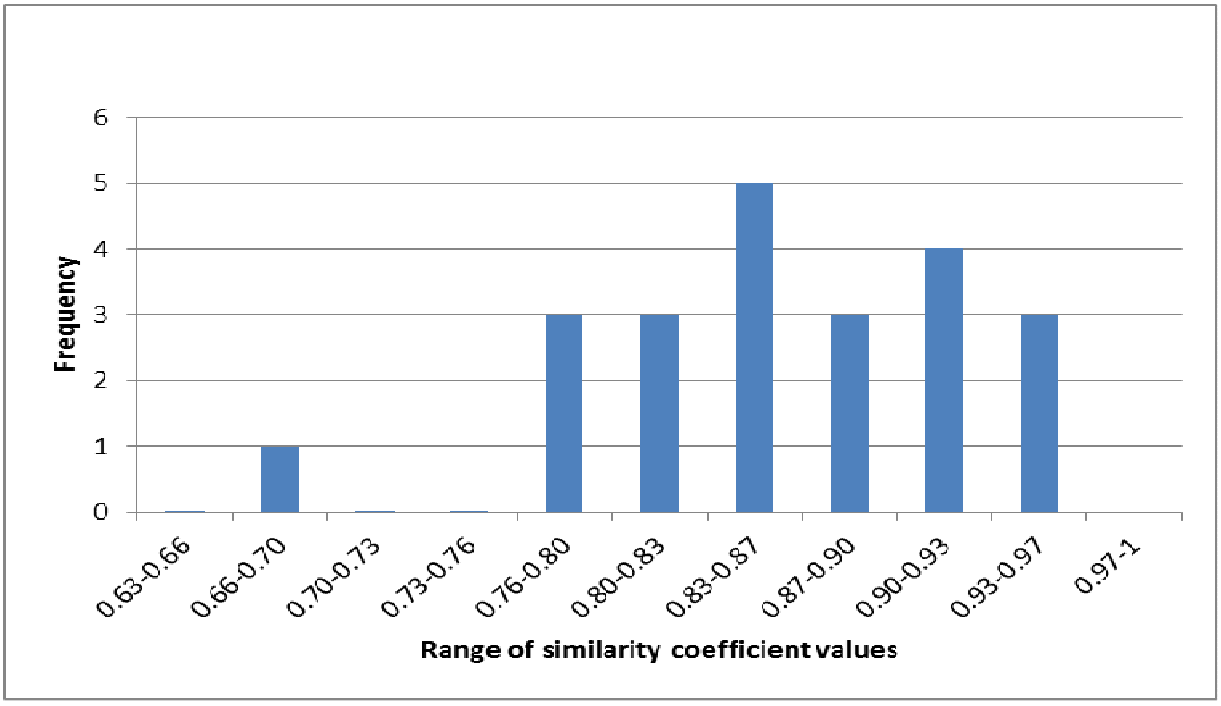


Fig .8. Frequency distribution of similarity coefficents in 25 taro accessions.

4.4.4. ITS2 sequences analysis

The Internal Transcribed Spacer 2 (ITS2) of the rDNA region of taro was amplified. This resulted in a 450 bp DNA fragment that was sequenced so as to detect polymorphisms (Fig. 9).

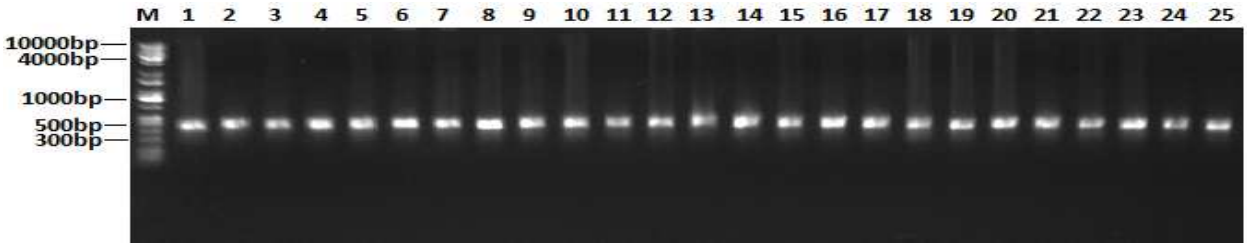


Fig.9. Amplified ITS2 region fragments of 25 taro samples (bp): base pairs, (M): Kapa Bio systems™ universal molecular weight marker, Lanes are numbered from 1- 25.

The sequencing chromatograms (Fig. 10) displayed strong peaks, minimal baseline noise and a few uncertainties ("N" and "S").

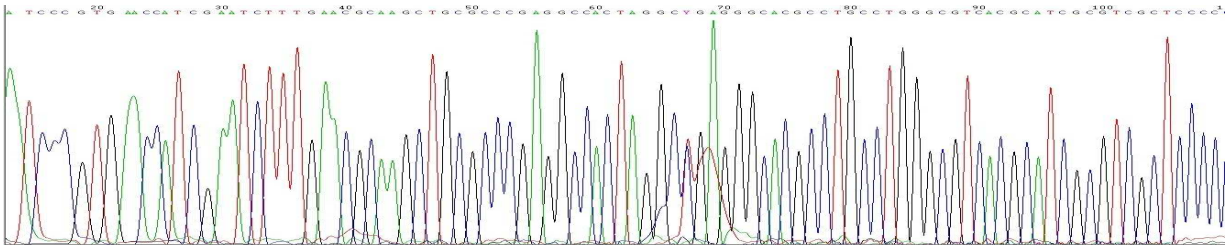


Fig. 10. ITS2 sequence chromatogram of the sample from Hluhluwe.

The ITS2 region of the accessions are represented in sequence length form for each of the taro accessions, showing the (G~C) contents and (A~T) contents in percentages form (Table 14).

Table 14. The ITS2 sequence lengths of the taro accessions and their (G~C) and (A~T) content in percentage form

Sample	Length (bp)	G-C content %	A-T content %
Hluhluwe	405	63.7	36.3
Maphumulo	405	66.2	33.8
Durban	405	64.2	35.8
Mbazwana	405	65.2	34.8
Willowvale	404	63.1	36.9
Ghana	405	66.7	33.3
Lusikisiki	404	63.4	36.6
Isipingo	405	62.2	37.8
Pietermaritzburg	405	64.2	35.8
Eshowe(1)	404	65.1	34.9
Eshowe(2)	405	64.4	35.6
Mangozi	399	67.7	32.3
Empangeni	403	66.5	33.5
Modderfontein	404	65.8	34.2
Tshwane(1)	406	65.0	35.0
Tshwane(2)	405	65.4	34.6
Tshwane(3)	406	64.9	35.1
Umbumbulu	405	65.4	34.6
Cocoindia	404	64.6	35.4
Jozini	404	64.6	35.4
Ntumeni	405	65.7	34.3
Nelspruit(Vanuatu)	403	66.5	33.5
Makatini res	401	67.6	32.4
Makatini	400	67.8	32.2
Nigeria	404	64.9	35.1

Analyses were conducted using the Maximum Composite Likelihood model (Tamura, Nei & Kumar 2004). Codon positions included were 1st+2nd+3rd+Noncoding. MEGA5 (Tamura, Dudley, Nei & Kumar 2007), to bring about the estimates of evolutionary divergence between sequences (Table 15).

Table 15. Estimates of evolutionary divergence between sequences

Hluhluwe																								
Maphumulo	0.254																							
Durban	0.078	0.273																						
Mbazwana	0.046	0.250	0.087																					
Willowvale	0.058	0.254	0.058	0.084																				
Ghana	0.246	0.038	0.273	0.250	0.254																			
Lusikisiki	0.063	0.250	0.093	0.058	0.093	0.254																		
Isipingo	0.081	0.301	0.072	0.072	0.090	0.297	0.075																	
Pietermaritzburg	0.072	0.250	0.105	0.066	0.099	0.250	0.041	0.090																
Eshowe 1	0.055	0.225	0.105	0.052	0.081	0.228	0.058	0.114	0.055															
Eshowe 2	0.087	0.266	0.130	0.084	0.127	0.273	0.055	0.099	0.052	0.081														
Mangozi	0.268	0.093	0.309	0.261	0.288	0.058	0.265	0.313	0.268	0.250	0.292													
Empangeni	0.254	0.041	0.273	0.258	0.258	0.055	0.258	0.309	0.265	0.236	0.289	0.105												
Modderfontein	0.096	0.293	0.111	0.058	0.130	0.297	0.066	0.081	0.063	0.084	0.075	0.308	0.301											
Tshwane1	0.084	0.247	0.152	0.090	0.120	0.254	0.090	0.159	0.081	0.049	0.096	0.269	0.254	0.114										
Tshwane2	0.069	0.232	0.124	0.078	0.093	0.235	0.084	0.143	0.081	0.035	0.102	0.254	0.236	0.114	0.041									
Tshwane3	0.084	0.304	0.081	0.102	0.087	0.296	0.108	0.063	0.127	0.136	0.133	0.321	0.300	0.118	0.175	0.159								
Umbumbulu	0.063	0.228	0.127	0.072	0.108	0.232	0.066	0.124	0.049	0.035	0.066	0.253	0.239	0.081	0.052	0.055	0.146							
Cocoindia	0.090	0.235	0.156	0.084	0.130	0.243	0.075	0.136	0.072	0.061	0.058	0.253	0.254	0.096	0.049	0.075	0.155	0.052						
Jozini	0.081	0.236	0.139	0.093	0.111	0.239	0.087	0.156	0.084	0.049	0.108	0.254	0.236	0.120	0.041	0.038	0.172	0.060	0.063					
Ntumeni	0.052	0.277	0.078	0.046	0.078	0.277	0.063	0.069	0.066	0.072	0.093	0.288	0.285	0.072	0.111	0.099	0.084	0.087	0.105	0.111				
Nelspruit	0.308	0.133	0.346	0.292	0.320	0.102	0.299	0.350	0.299	0.284	0.316	0.072	0.146	0.341	0.291	0.288	0.358	0.280	0.272	0.288	0.325			
Makatni res	0.268	0.093	0.309	0.261	0.288	0.058	0.265	0.313	0.268	0.250	0.292	0.000	0.105	0.308	0.269	0.254	0.321	0.253	0.253	0.254	0.288	0.072		
Makatni	0.268	0.093	0.309	0.261	0.288	0.058	0.265	0.313	0.268	0.250	0.292	0.000	0.105	0.308	0.269	0.254	0.321	0.253	0.253	0.254	0.288	0.072	0.000	
Nigeria	0.272	0.139	0.317	0.265	0.288	0.108	0.268	0.316	0.272	0.253	0.296	0.063	0.152	0.312	0.269	0.257	0.329	0.257	0.257	0.257	0.296	0.105	0.063	0.063

4.4.5. ITS2 generated sequence cluster analysis

The cluster analysis generated with the Maximum Likelihood method based on the Tamura-Nei model (Tamura & Nei 1993) from the ITS2 sequences of taro is shown in (Fig. 11).

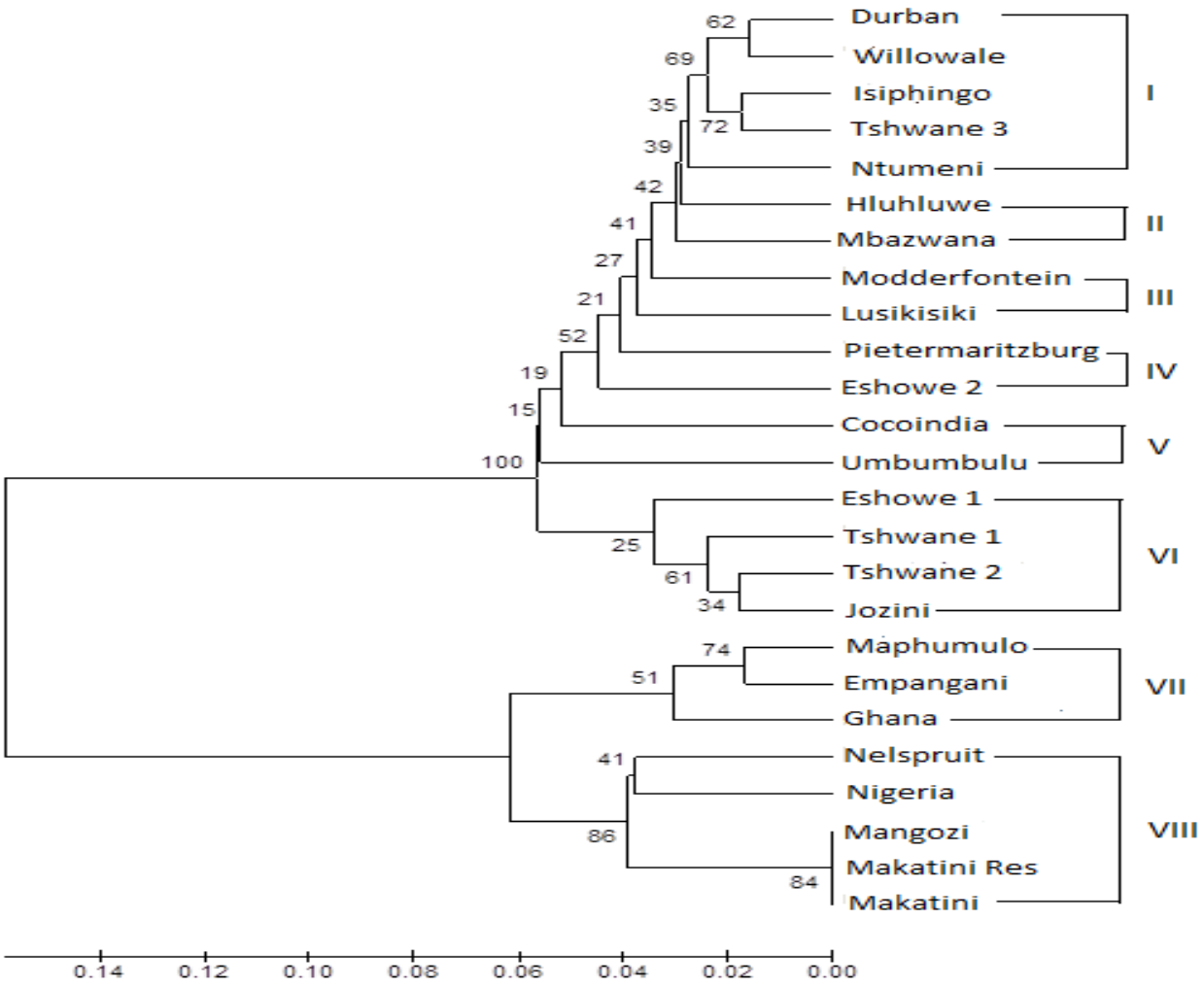


Fig. 11. Dendrogram derived from ITS2 sequence data

4.4.6. RNA secondary structure prediction

The structural elements identified in the 25 accessions of taro from the RNA secondary structure predictions. Counts of the structural elements for each accension is shown in (Table 16).

Table 16. The ITS2 secondary structure element counts in taro.

Name	Hairpin	Helix	Bulge loop	Multi-branch loop	Interior loop	Dangling end	Energy
Durban	3	3	5	1	1	1	-83.7
Empangeni	7	8	2	1	4	1	-157.09
Eshowe 1	10	7	1	1	11	1	-140.3
Eshowe 2	8	8	2	3	7	1	-166
Ghana	9	9	3	2	2	1	-154.76
Hluhluwe	6	6	5	2	11	1	-163.1
Isipingo	8	6	1	2	10	0	-103.52
Jozini	5	6	2	2	8	1	-173.7
Lusikisiki	8	7	4	4	9	1	-175.8
Makatini	8	9	4	2	10	1	-169.1
Mangozi	9	8	1	2	9	1	-162.5
Maphumulo	6	6	4	1	7	1	-165.6
Mbazwana	9	9	1	5	12	1	-206.8
Modderfontein	8	8	5	4	5	1	-110.88
Nelspruit	8	8	6	2	9	1	-156.4
Nigeria	6	8	8	8	6	1	-142.4
Ntumeni	6	7	1	3	12	1	-145.2
Pietermaritzburg	7	10	2	3	12	1	-165.76
Tshwane 1	8	5	4	3	8	1	-128.3
Tshwane 2	5	6	3	3	10	0	-115.87
Tshwane 3	10	11	3	4	8	1	-138.4
Umbumbulu	7	7	4	4	12	1	-166.8
Willowvale	6	6	2	1	6	1	-67.64
Cocoindia	10	8	1	3	11	0	-162.2
Makatini Res	8	8	1	1	8	1	-159.8

The predicted secondary structure of the taro sample from Maphumulo is shown in (Fig. 12). It highlights the conserved helix numbered I found in all 25 accessions of taro.

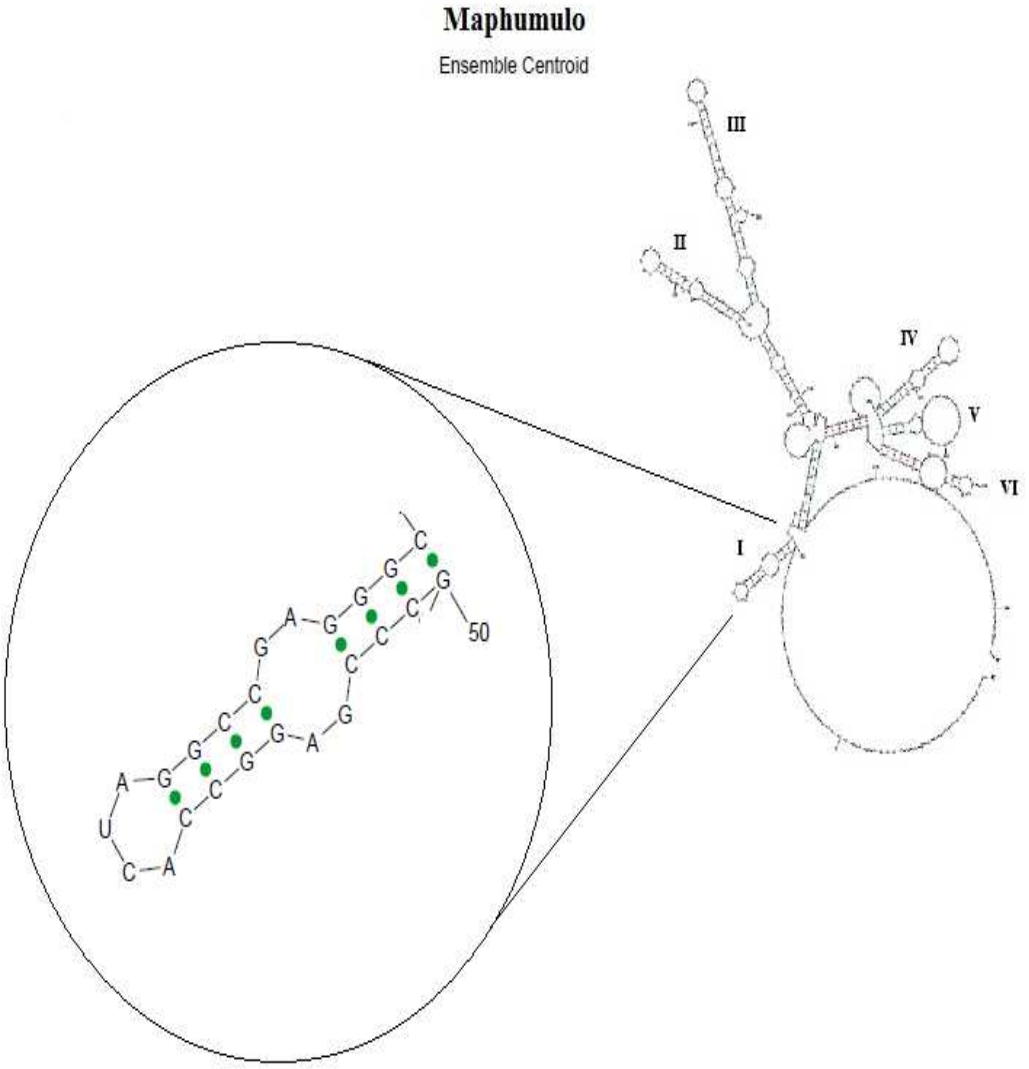


Fig. 12. The secondary structure of the sample Maphumulo showing the conserved helix numbered I found in 25 of the accessions of taro.

The (Fig. 13) shows the conserved helices (a) to (g) that were found to be recurring in some of the predicted secondary structures.

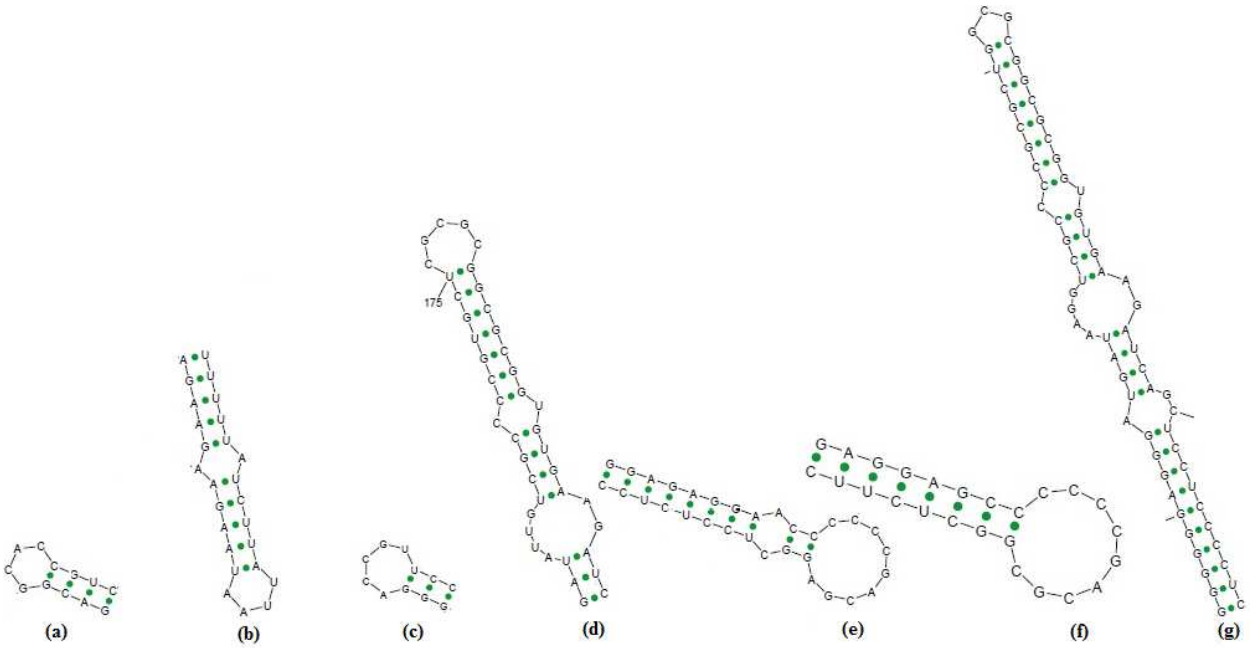


Fig. 13. The helices labeled (a) to (g) found in some of the secondary structures of the taro accessions.

CHAPTER (5) FIVE

DISCUSSION

5.1. Introduction

This chapter discusses and explains the data obtained from the morphological characterization, calcium oxalate quantification, RAPD analysis, ITS2 sequences and secondary structures derived from the ITS2 sequences.

5.2. Morphological characterization of taro

5.2.1. Quantitative traits

There was wide variability for the six numeric variables (lamina width, lamina length, petiole length, plant girth, number of leaves, number of lamina veins) measured in this study. For example, lamina width ranged from 4 cm to 30 cm, while the petiole length ranged from 7 cm to 44 cm. Beyene (2013) also found a wide range of lamina width and petiole length in his study of morpho-agronomical characterization of taro accessions in Ethiopia. There was a very high correlation between plant girth and lamina width (83%) and lamina width and lamina length (80%), (Table 6). A relatively high correlation was found between petiole length (petiole length in this study measured the height of the plant) and lamina length (70%) and plant girth and lamina length (71%). There appears to be some correlation between plant girth and lamina width. A high correlation between plant girth and lamina width was reported for East African highland banana (Nyombi 2010). High correlations between lamina width and lamina length was found in mulberry (*Morus* spp.), (Nderitu, Kinyua, Mutui & Ngode 2014). Opoku-Agyeman, Benneti-Lartey and Markwei (2004) also found high correlations between plant size and lamina length in cocoyam. It was interesting to find that the number of lamina veins was negatively correlated with lamina traits, petiole length and plant girth suggesting that this trait is probably under genetic control (Kang, Mizukami, Wang, Fowke & Dengler 2007). There was a poor correlation

between the number of leaves, leaf sizes and petiole length (Table 6). Similarly, there was a moderate level of relationship between leaf number and plant girth (48%).

Principal Component Analysis (PCA) showed that most of morphological variance was explained by the first two principal components with eigen values greater than 1 (Table 7). Component 1 explained 54% of the variance while component 2 explained 20 % of the variance. Most of the morphological variation was due to lamina length, petiole length, lamina width and plant girth.

5.2.2. Qualitative traits

The green lamina colour was highly uniform among all the accessions except for the sample from Nelspruit (Vanuatu) which had a purple lamina. The green petiole colour was also predominant in all the accessions except for those from Nigeria and Durban. The two plants, however, differed in other morphological characteristics making these two accessions easy to identify.

5.3. Calcium oxalate crystal analysis

There was large variation in the number of raphides containing calcium oxalates crystals per cell among the accessions ranging from 27 ±12 in the accession Ntumeni to 1150 ±104 in the Modderfontein accession (Table 10). There was a highly significant difference ($P = 0.001$) in the number of raphides among the accessions (Table 11). This is the first report on the number of raphide crystals in taro. Although there are no studies that have quantified the number of raphides in taro, there are reports of accessions with low and high levels of acridity that is due to the concentration of raphides in plants (Matthews 2010). The median value for the number of raphides among the accessions in this study is 180. On this basis, this study proposes that accessions with less than 180 raphides per cell be regarded as plants with the low acridity. This

study has identified twelve taro accessions as having high levels of acridity ranging from 220 to 1150 raphides per cell (Table 10). On this basis, accessions with low counts of raphides including Ntumeni, Makatini, Empangeni, Cocoindia, Nelspruit (Vanuatu), Eshowe 1, Tshwane 2, Tshwane 3, Makatini Res, Isipingo, Hluhluwe and Jozini, could be recommended for leaf consumption. It is known that farmers favour cultivars with low acridity (Singh, Hunter, Iosefa, Okpul, Fonoti & Delp 2010). It would be interesting to compare the content of raphides in the leaves and corms of taro since these are the most commonly used parts of the plant.

5.4. RAPD data

In this study, nine out of the twenty RAPD primers produced amplification patterns that were informative (Table 13). A total of 1158 bands were scored with the nine primers, of which 719 bands (63.6%) were polymorphic (Table 13). A typical RAPD amplification pattern is shown in (Fig. 6). The 25 taro accessions showed a high level of genetic diversity as observed from the Jaccard's similarity coefficients that ranged from 0.63 to 1.00 in the RAPD dendrogram (Fig. 7) and the frequency distribution of similarity coefficents (Fig. 8). High levels of genetic variation were also reported in a number of studies in taro. Lakhanpaul, Velayudhan and Bhat (2003) reported a high level of genetic diversity of taro in India using RAPD with thirteen random primers and 32 taro accessions. In a RAPD study, Mishra, Sharm and Misra (2008) found high levels of genetic diversity in 10 accessions of taro collected from different parts of India. Similarly, high levels of genetic diversity were identified among 46 taro accessions in China (Maolin, Rugang, Zhou Mingde, Eyzaguirre & Ayad 2010) using RAPD analysis.

It is generally assumed that clonally propagated crops have a low genetic diversity (Eckert 1999). However, many clonally propagated crops have revealed high levels of genetic diversity. this has been seen in olive tree *Olea europea,* (Mekuria , Collins & Sedgley 1999), henequen

Agave fourcroydes (Gonzalez, Alemana & Infante 2003), banana *Musa acuminata* Colla (Mukunthakumar , Padmesh , Vineesh , Skaria , Kumar & Krishnan 2013) .It is believed that this diversity may be due to a number of mechanisms including mutations, translocations, chromosomal deletions and duplications (Nath, Senthil, Hegde, Jeeva, Misra, Veena & Raj 2013). Different chromosome numbers may also have a big row to play in the overall variation and high genetic diversity seen in this study. Chromosome numbers of South African taro have not been identified. Chromosome numbers reported for taro in other studies outside South Africa include 2n = 22, 26, 28, 38, and 42 (Onwueme 1999). The disparity in the numbers may be due to the fact that taro chromosomes are liable to unpredictable behavior during cell divisions (Onwueme 1999).

RAPD analysis separated the accessions of this study into three main clusters (Fig. 7). Each main cluster was divided into subclusters. The first main cluster (1a) was composed of two groups of accessions. One group consisted of five accessions from Hluhluwe, Mbazwana, Eshowe 1, Eshowe 2, and Empangeni. The close clustering of these accessions from this locality is interesting. These locations lie within a radius of 156 km in the province of KwaZulu-Natal. The accessions were very similar morphologically (Table 5). The similarity of the accessions may be due to the exchange of planting material among the farmers in the area. However, the number of raphide crystals differed among the accessions with three, Hluhluwe, Empangeni and Eshowe 1 being in the low group and two, Mbazwana and Eshowe 2, falling in the high group. It was interesting to note that the two accessions from Eshowe, while being morphologically similar, fell into different groups with regards to the number of crystals (Table 10). Accessions coming from the same locality may be expected to have similar crystal counts. However,

environmental factors such as drought or nutrient stress during the growing season can affect the acridity of the same cultivar in different environments (Onwueme 1999).

The second group consisted of accessions Tshwane1, Tshwane 2, Nigeria and Ntumeni. While the clustering of the 2 accessions from Tshwane (Gauteng Province) was expected, the Ntumeni accession was obtained from KwaZulu-Natal. If we assume that the accession, Nigeria, is truly of Nigerian origin, then it is probable that the accession was introduced to Tshwane by Nigerian immigrants. This is a unique accession with a purple petiole, very high plant height and high raphide crystal count.

Cluster Ib was composed of accessions from Lusikisiki, Isipingo and Pietermaritzburg with the former two accessions being in a sister group relationship. Isipingo is located in Southern Kwazulu Natal while Lusikisiki is in the eastern part of the Eastern Cape. Both locations are separated by a distance of 185 kms. The close grouping of the Isipingo and Lusikisiki accessions may be evidence of sharing of plant material between the two provinces. This may also explain the grouping of the accession from Pietermaritzburg with the former two accessions.

The rest of the accessions in this study did not group according to their areas of origin. Several studies have also reported lack of correlation of geographical origin and clustering of taro (Irwin, Kaufusi, Banks, De la pena & Cho 1998; Lakhanpaul et al. 2003, Sharma et al. 2008; Nath et al. 2013). Some of the accessions such as the one from Durban (purple petiole) and Nelspruit (purple leaf) had distinct morphological characters but still revealed that they were genetically different.

Lebot, Hartati, Hue, Viet, Nghia, Okpul, Pardales, Prana, Thongjiem, Krieke, VanEck, Yap and Ivancic (2010), explained that somatic mutations occur constantly in taro and are retained. Therefore cultivars that appear to be morphologically similar may be genetically different. The

phenotypic variation found in Polynesian taro was also explained by the accumulation of somatic mutations through a very intense rate of clonal propagation (Lebot & Aradhya 1991). High morphological variation between cultivars is not a guarantee for a high genetic variation (Kreike et al. 2004).

5.5. ITS2 sequence analysis and dendrogram

Amplification of the internal transcribed spacer (ITS2) of the rDNA of taro produced a 450 bp fragment (Fig. 9). The length of the ITS2 regions was fairly uniform among the accessions with the exception of Mangozi (Table 14). The G~C content of the ITS2 region ranged from 62.2% - 67.8% while the A~T content varied from 32.3 - 36.9% (Table 14). The aligned sequence data for the ITS2 (ANNEXURE A) region had 403 nucleotides. Among these, 160 were variable, 242 were conserved and 127 were informative. The latter was used for parsimony analysis.

The clustering based on the ITS2 sequences generated a tree that was divided into eight groups. With the exception of group VI, parts of group VII and group VIII, the dendrogram (Fig. 11) derived from the ITS2 sequence data did not group the accessions according to their points of locality. The close affinity of accessions from Tshwane1 and Tshwane 2 (group VI) is explanable by their locality. A similar reason may be extended for the two accessions from Maphumulo and Empangeni in group VII. Despite the purple stem of the accesion from Nigeria and the purple leaf of the accession Nelspruit, they group together in group VIII. The close grouping of the accessions from Makatini, Makatini Res and Mangozi may be explained by the proximity of these locations.

5.6. Secondary structure prediction

The secondary structures of the ITS2 region were analysed based on variable and conserved elements in all 25 accessions of taro. The 25 accessions had an average of seven helices, seven

hairpins, three bulge loops, three multi-branch loop, eight interior loops, and a dangling end (Table 16). In general, these structures conform to the proposed four helices ITS2 model for flowering plants and green algae (Mai & Coleman 1997), yeast and vertebrates (Joseph et al. 1999), Scleractinian corals (Chen, Chang, Wei, Chen, Lein, Lin, Dai & Wallace 2004), Lepidteran species (Kuracha, Rayavarapu, Kumar & Rao 2006), *Anopheles culicifacies* (Dassanayake, Gunawardene, Nissanka & Silva 2008) and eukaryotes as a whole (Schultz, Maisel, Gerlach, Muller & Wolf 2005). However, this study showed that most of the accessions had additional helices ranging in number from 5 to 11. Similar finding were reported for corals (Chen et al. 2004), and in bivalve subfamily Pectininae (Salvi, Bellavia, Cervelli & Mariottini 2010). On the contrary, in cnidarians various helices were found to be missing (Oliveiro, Barco, Modica, Richter & Mariottini 2009). It was suggested that closely related taxa with very similar primary sequences can result in/exhibit different structures (Hershkovitz & Zimmer 1996). One of the distinct hallmarks of the core secondary structure is the highly conserved motif of 5' -UGGU- 3' and the deviation 5'- UGG- 3' found in all eukaryotes. This was also found in the predicted structures of taro at approximately nucleotide positions (291-293) (ANNEXURE A). The ITS2 secondary structures of the 25 taro accessions generated a variety of forms ranging from simple to complex structures (ANNEXURE B). The simplest secondary structure with three helices was found in the accession from Durban while the accession Tshwane 3 produced a complex structure with 11 helices, 4 multi-branched loops, 3 bulge loops and 8 interior loops. The predicted secondary structures of the ITS2 among the 25 taro accessions were quite variable and did not exhibit a common model based on topology. However, there was a common motif in all the accessions 5'-GCCGAGGCCACUAGGCCGAGGGC-3' at approximately nucleotide positions (48-73). This motif was found in all the taro accessions in helix 1(Fig. 12). This is the

first study to identify and report such a recurring motif in taro. This motif conserved in all the accessions is probably due to a functional significance of the region. Van Nues, Rientjes, Morré, Mollee, Planta, Venema and Raué (1995) suggested that the nature of the conserved motifs may probably have a function in the regulation of the transcription of active ribosomal subunits as this provides structural elements necessary for the correct pre-rRNA processing.

Besides the common motif found in all accessions at positions (48-73), some other motifs were also found in the accessions, though not in all 25 accessions (Fig. 13). Whilst some of the motifs were unique to some accessions others were found to be recurring in more than one accession. Table 17 shows the accessions that exhibit the motifs shown in Figure 13 (a) to (g). The accessions marked with an asterisk (*) in (Table 17) only have one motif in their structure.

Table 17. The conserved motifs (a) to (g) found in taro secondary structures.

(a)	(b)	(c)	(d)	(e)	(f)	(g)
Mangozi*	Modderfontein*	Willowvale*	Ntumeni*	Tshwane1	Isipingo	Cocoindia
Makatini *	Eshowe2*	Isipingo	Lusikisiki	Tshwane2	Hluhluwe	Jozini
MakatiniRes*	Lusikisiki	Hluhluwe	Mbazwana	Eshowe1	Eshowe1	
Nigeria*	Tshwane1	Eshowe1				
	Mbazwana	Mbazwana				
	Pietermaritzburg	Tshwane1				
		Cocoindia				
		Pietermaritzburg				
		Jozini				

The rest of the accessions had two or more other motifs within their structures inclusive of the common motif in all the accessions, an example is Mbazwana which exhibited motif (b),(c) and (d) in its structure. The structural motifs may be used as a way of distinguishing taro accessions. Though an argument maybe made that subsistence farmers at Umbumbulu and Ndwedwe are able to distinguish local taro varieties by means of morphological characteristics as justified by (Shange 2004), but the ITS2 region and the prediction of secondary structures becomes a more

reliable measure of diversity and identification compared to morphological plant characters, as more limitations are evident with morphological diversity assessments, these include; differential heritability's, pleiotropic and epistatic effects and more importantly the problem of most genetic variation being hidden and not apparent at the phenotypic level, hence morphologically similar material may in fact be genetically quite different (Lebot et al. 2010) and morphologically diverse material may be very similar genetically (Lebot & Aradhya 1991).

The secondary structure of the ITS2 regions appears to be a significant barcoding marker as well as a taxonomic tool in these studies. This is the first study that predicted the ITS2 secondary structures in taro. The secondary structures of taro were unique to each accession. The structures presented in this study are capable of being used as tools for the identification of each of the 25 accessions of taro.

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CHAPTER (6) SIX

CONCLUSION AND RECOMMENDATIONS

6.1. Conclusion

The study looked at the morphological diversity of the aerial portions of taro, the quantification of raphides containing calcium oxalate crystals, the genetic diversity of taro accessions using RAPD and the ITS2 region, and finally the prediction of the secondary structure models of the ITS2 sequences in taro.

Morphological characterisation showed that most of the variation was found in four characters: lamina length, petiole length, lamina width and plant girth with principal component loadings ranging from (0.693) to (0.938), explaining 54% of the variance. Overall, this study indicated that the aerial parts of taro are variable for most quantitative characters. Variation is an indication for the potential of genetic improvement through selection and breeding (Beyene 2013).

The number of raphides was able to divide the 25 accessions into two groups, one with relatively low counts and the other with high counts. Ntumeni had the lowest raphide count of only 27 ±12 and Modderfontein had the highest count with 1150 ±104 raphides. Twelve accessions in the study have been proposed as having low calcium oxalate crystals. The importance of knowing the accessions that have low calcium oxalate crystals is useful for breeding. Low acridity accessions can be promoted for culinary purposes.

RAPD data separated the accessions into three main groups that were further divided into five subgroups. The accessions did not group according to geographical location. The ITS2 sequence generated clustering patterns that were similar to that obtained from RAPDs.

Growers currently differentiate taro accessions by morphology and naming them according to where they are located. This type of identification creates problems as growers give two different accessions the same name resulting into duplicates (Beyene 2013). This also provides a database

for taro breeders as well to make informed choices in the selection of parental accessions for offspring's to obtain superior varieties.

The variation in the ITS2 secondary structure of taro included one common motif that was present in all 25 accessions. Some motifs were only present in some accessions. The discovery of these exclusive motifs strengthens the potential of the ITS2 region as a taxonomic marker and provides the means of identifying each of the 25 accessions of taro. The ITS2 has been used for genus and species identification (Chen, Yao, Han, Liu, Song, Shi, Zhu, Ma, Gao, Pang, Luo, Li, Li, Jia, Lin & Leon 2010). The ITS2 region may be a powerful barcode for identification of taro.

6.2. Recommendations

The following recommendations are made for further studies of taro:

The morphological observations of the plants used in this study were obtained from single plants grown in the greenhouse. It would be interesting to observe the characteristics from field grown plants. In this way plants could be grown in duplicates or triplicates. This would allow statistical analysis of the morphological variation. Mandal, Mukherjee, Mandal, Tarafdar and Mukharjee (2013).

A comparison of the aerial morphological traits and corm characteristics would be important in gauging yield traits (Bradshaw 2010).

Growth in different types of soil with varying alkalinity and acidity would ascertain the effect soil types have on the quantity of calcium oxalate crystals within the plant. It is reported that environmental stress conditions can affect acridity in taro (Libert & Franceschi 1987; Onwueme 1999).

The quantification of the calcium oxalate crystals was done by counting the raphides under a bright field microscope from cleared leaf sections. A future study may also employ the use of

extraction techniques for soluble and insoluble oxalates (Libert & Franceschi 1987) and quantification of the crystals with High-Performance Liquid Chromatography (HPLC). The HPLC has been used in assessing organic acids such as calcium oxalate in spinach (Savage, Vanhanen, Mason & Ross 2000). This study assessed the number of raphidses only in the leaves of the taro accessions. It would interesting to examine the number of crystals in the leaves and corms since these are the two parts of the plants that are normally consumed.

The study used 20 primers for the RAPD analysis. Increasing the number of primers would be helpful in broadening the understanding and extent of genetic diversity in taro.

Compensatory base changes (CBC's) can be accessed with the use of a CBC analyzer. CBCs occur in a paired region of a primary RNA transcript when both nucleotides of a paired site mutate, while the pairing itself is maintained. Analysis of these CBCs in the ribosomal RNA (ITS2) secondary structure has been used to successfully verify the taxonomy of closely related species. This is significant in the sense that discrimination between two closely related species by a single CBC can be achieved (Muller, Philippi, Dandekar Schultz & Wolf 2007). CBCs in the ITS2 secondary structures are found to correlate strongly with distinct biological species. One can use this molecular indicator for determining at least the minimal number of distinct species from a set of ITS2 secondary structures of a clade (Muller et al. 2007). This study was not able to identify a working computer programme for the CBC analysis. CBC analysis may be able to verify species classification in taro.

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ANNEXURE A: Multiple sequence alignment of the 25 accessions of taro

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Lusikisiki            ---UGGCGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 56
Eshowe2               --UCGCUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Pietermaritzburg      --GGAGUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Modderfontein         --GCAGUGAUUGCAGA-UCCCGUGA-CCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 56
Tshwane2              --GGAGCGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Jozini                ---UAGCGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 56
Tshwane1              -GCAGGUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 58
Cocoindia             ---GAGCGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 56
Umbumbulu             --GGAGCGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Eshowe1               --AGAGUGAUUGCAGA-UCCCGUGA-CCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 56
Mbazwana              --GCGGUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Ntumeni               UAGCGG--AUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Hluhluwe              --UGAGUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Durban                ---CGGCGAUUGCAGAAUCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Willowvale            ---UAGCGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 56
Isipingo              --UGAGUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Tshwane3              -GGADGUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 58
Maphumulo             --AGAGUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Empangeni             ---CGGUGAUUGCAGA-UCCCGUGA-CCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 55
Ghana                 ---UAGCGAUUGCAGAAUCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Mangozi               -UAGAGG-AUUGCAGA-UCCCGUGA-CCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 56
Makatini              --AGAGGGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
MakatiniRes           --UGGGUGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
Nigeria               ---GAGCGAUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 56
Nelspruit             -UCGCGC-AUUGCAGA-UCCCGUGAACCAUCGAAUCUUUGAACGCAAGCUGCGCCCGAGG 57
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Lusikisiki            CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 116
Eshowe2               CCACUAGGCCGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Pietermaritzburg      CCACUAGGCCGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Modderfontein         CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 116
Tshwane2              CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Jozini                CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 116
Tshwane1              CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 118
Cocoindia             CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 116
Umbumbulu             CCACUAGGCCGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Eshowe1               CCACUAGGCCGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 116
Mbazwana              CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Ntumeni               CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Hluhluwe              CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Durban                CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Willowvale            CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 116
Isipingo              CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Tshwane3              CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 118
Maphumulo             CCACUAGGCCGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Empangeni             CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 115
Ghana                 CCACUAGGCCGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Mangozi               CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 116
Makatini              CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
MakatiniRes           CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
Nigeria               CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 116
Nelspruit             CCACUAGGCUGAGGGCACGCCUGCCUGGGCGUCACGCAUCGCGUCGCUCCCCCGACUCCC 117
                      ********* ************************************************

Lusikisiki            CCCAGAAG-----GGACCGUUCCGGAAGGGGGGGGGGGGGAGUGAAGAUAUUGUCGCCCCG 171
Eshowe2               CCCAGAAG-----AGACCGUUCCGUAAGGGGGGGGGGGGGAGUGAAGAGAUUGUCGCCCCG 172
Pietermaritzburg      CCCAGACG-----GGACCGUUCCGUAAGGGGGGGGGGGGGAGUGAAGAUAUUGUCGCCCCG 172
Modderfontein         CCCAAAAG-----GGACCGGUCCGCCAGGGGGGGGGGGGGAGUGAUGAUAUUGUCGCCCCG 171
Tshwane2              CCCAGACG-----GGACCGUGCCGUAAGGGGGGGGGGGGGAGGGAUGAUAGGGUCGCCCCG 172
Jozini                CCCAGACG-----GGACCGUUCCGUCCGGGGGGGGGGGGGAGGGAUGAUAAGGUCGCCCCG 171
Tshwane1              CCCAGACG-----GGACCGUUCCGUCAGGGGGGGGGGGGGAGGGAUGAUAGGGUCGCCCCG 173
Cocoindia             CCCAGAAG-----GGACCGCUCCGUCAGGGGGGGGGGGGGAGGGAUGAGAAGGUCGCCCCG 171
Umbumbulu             CCCAGAAG-----GGACCGUUCCGUCAGGGGGGGGGGGGGAGUGAUGAUAGGGUCGCCCCG 172
Eshowe1               CCCAGACG-----GGACCGUUCCGGCAGGGGGGGGGGGGGAGGGAUGAUAGGGUCGCCCCG 171
Mbazwana              CCCAGAAG-----GGACCGUUCCGGAAGGGGGGGGGGGGGAGGGAAGAUAGGGUCGCCCCG 172
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Ntumeni              CCCAGACG-----GGACCGUUCCGGAAGGGGGGGGGGGGGAGUGCUGAUAGGGUCGCCCCG 172
Hluhluwe             CCCAGACG-----GGACCGUUCCGGCAGGGGGGGGGGGGGAGUGAAGAUAGGGUCGCCCCG 172
Durban               CCCAGAAG-----GGACCGUUCCGGAAGGGGGGGAGGGGGUGUGAAGAUUGUGUCCCCCCG 172
Willowvale           CCCAGACG-----GGACCGUUCCGGAAGGGGGGGAGGGGGUGUGAAGAUAGGGCCGCCCCG 171
Isipingo             CCCAAAAG-----GGACCGUUCCGGAAGGGGGGGAGGGGGUGUGAAGAUAUUGCCCCCCCG 172
Tshwane3             CCCAGAAC-----GGACCGUUCCGGCAGGGGGGGAGGGGGUGUGAAGAUAGGGCCCCCCCG 173
Maphumulo            CCCAGACGAGACCGCACCGUCCGCGAGGGGGGAAAGGGAUGCUGAGAUUGGCCCGCCGUG 177
Empangeni            CCCAGACGAGACCGCCCCUUCCGCGUGGGGGGGAAGGGAUGCUGAGAUUGGCCCGCCGUG 175
Ghana                CCCAGACGAGACCGCACCGUCCGCGAGGGGGGCGAGGGAUGCUGAGAUUGGCCCACCGUG 177
Mangozi              CCCAGACG-----GCACCGUCCGUGAGGGGGGCGAGGGAUGCGGAGAUUGGCCCACCGUG 171
Makatini             CCCAGACG-----GCACCGUCCGUGAGGGGGGCGAGGGAUGCGGAGAUUGGCCCACCGUG 172
MakatiniRes          CCCAGACG-----GCACCGUCCGUGAGGGGGGCGAGGGAUGCGGAGAUUGGCCCACCGUG 172
Nigeria              CCCAGACG-----GCACCGUCCCUGAGGGGGGGGAGGGAUGCGAAGAUUGGACCACCGUG 171
Nelspruit            CCCAGAGG-----GCACCGUCCGGUAGGGGGGGGAGGGAUGGAGAGAUUGGUCCACCGUG 172
                     **** *          **   *     *****   ***  *    **      * **  *

Lusikisiki           UGCUCGCGCGGCGCGGUGUGAAGAUCAGCUCCUCCCCCCCCCGCGCGAGCAGCGCGAGUGG 231
Eshowe2              UGCUCGCGCGCCGCGGUGUGAAGAUCUGCUCCCCCCCCCCCCGGGGGAGCAGCGCGAGGGG 232
Pietermaritzburg     CGCUCGCGCGCCGCGGUGUGAAGAUCAGCUCCCCCCCCCCCCGCGCGAGCAGCGCGAGGGG 232
Modderfontein        UGCUCGCGCGCCGCGGUGUGAAGAUCUCCUCCCCCCCCCCCCGCGCGAGCAGCGCGAGGGG 231
Tshwane2             CGCUCGCGCGGCGCGGUGUGAAGAUCAGCUCCUCCCCUCCCGCGAGACCAGCGCGGGGGG 232
Jozini               CGCUGGCGCGGCGCGGUGUGAAGAUCAGCUCCUCCCCUCCCGCGGGGCCAGCGCGGGGGG 231
Tshwane1             CGCUGGCGCGGCGCGGUGUGAUGAUCAGCUCCUCCCCUCCCGCGGGACCAGCGCGGGGGG 233
Cocoindia            CGCUGGCGCGGCGCGGUGUGAAGAUCAGCUCCUCCCCUCCCGGGGGAGCAGCGCGAGGGG 231
Umbumbulu            CGCUCGCGCGCCGCGGUGUGAAGAUCAGCUCCUCCCCCCCCCGCGCGUGCAGCGCGAGGGG 232
Eshowe1              CGCUCGCGCGGCGCGGUGUGAAGAUCAGCUCCUCCCCCCCCCGCGAGAGCAGCGCGAGGGG 231
Mbazwana             CGCUCGCGCGGCGCGGUGUGAAGAUCUGCUCCUCCCCCCCCCGGGAGAGCAGCGCGAGGGG 232
Ntumeni              CGCUCGCGCGGCGCGGUGUGAAGAUCUGGUCCCCCCCCCCCCGGGCGAGCAGCGCGAGGGG 232
Hluhluwe             CGCUCGCGCGGCGCGGUGUGAAGAUCUGCUCCUCCCCCCCCCGGGAGAGCAGCGCGAGGGG 232
Durban               UGCUCGCGCGGCGCGGUGUGAAGAUCUGCUCCUCCCCCCCCCGCGAGAACAGCGAGAGGGG 232
Willowvale           CGCUCGCGCGGCGCGGUGAGAAGAUCAGCUCCUCCCCCCCCCGCGAGAACAGCGAGAGGGG 231
Isipingo             UGCACGCGCGCCGCGGUGUGAAGAUCUGCUCCUCCCCCCCCCGGGAGAGCAGCGAGAGGGG 232
Tshwane3             UGCACGCGCGGCGCGGUGUGAAGAUCUCCCCCCCCCCCCCCCGGGAGAGCAGCGAGAGUGG 233
Maphumulo            CCCCCGCGCGGUGGGCUGAUCAGCUCGGCCCCUCCC--GCCGGGCGAGCACGGGGAGUGG 235
Empangeni            CCCGCGCGCGGUGGGCUGAUCAGCUCGGCCCCUCCG--GCCGGGCCAGCACGGGGAGUGG 233
Ghana                CACGCGCGCGGUGGGCUGAUCAGCUCGGCCCCUCCC--GCCGGGCGAGCACGGGGAGUGG 235
Mangozi              CACGCGCGCGGCGGGCUGAAGAGCUCGGCCCCUCCC--GCCGGGCGAGCACGGCGAGUGG 229
Makatini             CACGCGCGCGGCGGGCUGAAGAGCUCGGCCCCUCCC--GCCGGGCGAGCACGGCGAGUGG 230
MakatiniRes          CACGCGCGCGGCGGGCUGAAGAGCUCGGCCCCUCCC--GCCGGGCGAGCACGGCGAGUGG 230
Nigeria              CACGCGCGCGGCGGGCUGAAGAGAUCGGCCCCUCCC--CCCGGGCGAGCACAGCGAGUGG 229
Nelspruit            CACGCGCGCGGGGGGCUGAAUAGCUCGGUCCCUCCC--GCCGGGCGAGCACGGCCAGUGG 230
                       *  *****  * * **    * **    ** **    *** *    **  *   * **

Lusikisiki           UGGUAUAACACUCCUCAUCGUCGUGGCGCACCCCCGCGCGCGGCGAGGGUGGACCCACCA 291
Eshowe2              UGGUAUAACACUUCUCAUCGUCGUGGCGCACCCCCGCCCGGGGGGAGGGAGGACCCACCA 292
PietermariUzburg     UGGUAUAACACUUCUCAUCGUCGUGGCACACCCCCGCGCGCGGCGAGGGUGGACCCACCA 292
Modderfontein        UGGUAUAACACUUCUCCUCGCCGUGGCACACCCCCGCGCGCGGCGAGGGUGCACCCACCA 291
Tshwane2             UGGUAUAACACUUCUCAUCGUCGUCGCGCCCCCCCCGCGCGCGGCGAGGAUGGACCCACCA 292
Jozini               UGGUAUAACACUUCUCAUCGUCGUCGCGCACCCCCCGCGCGCGGCGAGGAUGGACCCACCA 291
Tshwane1             UGGUAUAAUACUUCUCAUCGUCGUCGCGCCCCCCCCCCCCGCGGCGAGGAUGGACCCACCA 293
Cocoindia            UGGUAUAACACUUCUCAUCGUCGUGGCGCACCCCCGCCCGGGGCGAGGAAGGACCCACCA 291
Umbumbulu            UGGUAUAACACUUCUCAUCGUCGUGGCGCACCCCCCGCGCGCGGCGAGGAUGGACCCACCA 292
Eshowe1              UGGUAUAACACUUCUCAUCGUCGUGGCGCACCCCCCGCGCGCGGCGAGGAUGGACCCACCA 291
Mbazwana             UGGUAUAACACUUCUCAUCGCCGUGGCACACCCCCGCGCGCGGCGAGGGUGCACCCACAA 292
Ntumeni              UGGUAUAACACUUCUCAUCGGCGUGGCACACCCCCGCGCGCGGCGAGGGUGCCCCCACAA 292
Hluhluwe             UGGUAUAACACUUCUCAUCGUCGUGGCACACCCCCGCGCGCGGAGAGGGUGAACCCACAA 292
Durban               UGGUAUAACUCUUCUCGUCGCCGUGGCACACCCCCGCGCGAGGCGAGGGUGACCCCACAA 292
Willowvale           UGGUAUAACACUUCUCAUCCUCGUGGCACACCCCCGCGCGAGGAGAGAGUGCCCCCACAA 291
Isipingo             UGGUAUAACACUUCUCAUCGCCGUGGCACACCCCCGCGCGAGGAGAGGGUGCACCCACAA 292
Tshwane3             UGGUAUAACACUUCUCGUCGGCGUGGCACACCCCCGCGCGAGGAGAGGGUGCCCCCACAA 293
Maphumulo            UGGAAUAACUCUCAUCCUCGCCGUGGCCCACCCCCGCACG---CGAGGACGGACCGACCA 292
Empangeni            UGGAAUAACUCUCAUCGUCGCCGUGGCCCACGCCCGCGCG---CGAGGAUGGACCGACCA 290
Ghana                UGGAAUAACACUCAUCGUCGCCGUGGCACACGCCCGCGCG---CGAGGACGGACCGACCA 292
Mangozi              UGGAAUAACACUCAUCGUCGCCGUGGCACACGCCCGCGCG---CGAGGACGGACUGACCA 286
Makatini             UGGAAUAACACUCAUCGUCGCCGUGGCACACGCCCGCGCG---CGAGGACGGACUGACCA 287
MakatiniRes          UGGAAUAACACUCAUCGUCGCCGUGGCACACGCCCGCGCG---CGAGGACGGACUGACCA 287
Nigeria              UGGAAUAACACUCAUCUCCUCCGUGGCACGCGCCCGCGCG---CGAGGACGGACUGACCA 286
Nelspruit            GGGAA-GGCACUCAUUGUUCCCGUGGCACACGCCCGCUCG---CGAGGACGGACCGACCA 286
                      ** *     **  *      *** ** * * *** * **    ***   *  *  ** *

Lusikisiki           AGAAAACACUCCCGAGAGAGAAGAGGAGCCCCCCGACGAGGCUCUUCUCCCACCACCACG 351
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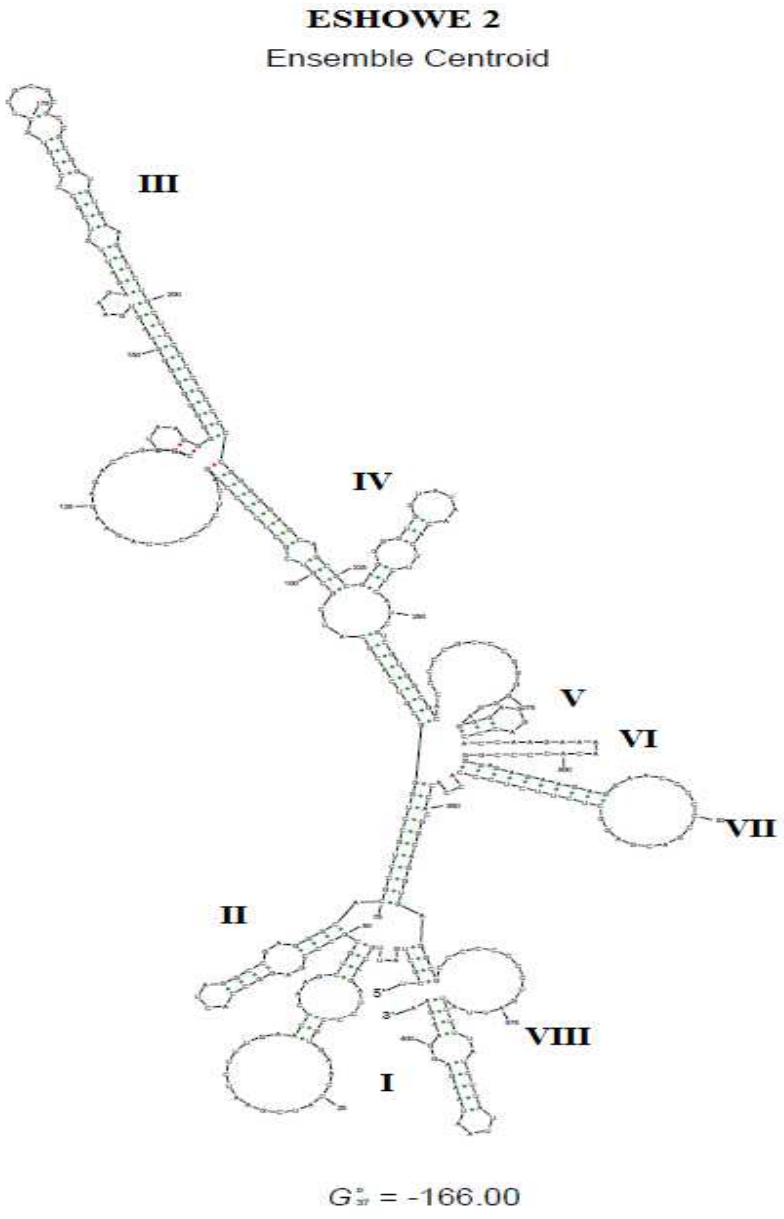
Eshowe2              AGAAAACACCCCGGGGAGAGAAGAGGAACCCCCCGACGAGGCUCUUCUCCCACCACCACG 352
Pietermaritzburg     AGAAAACACUCCCGUGCGAGAAGAGGAACCCCCCGACGAGGCUCUUCUCCCACCACCACG 352
Modderfontein        AGAAAACACCCCCGGGGGAGAAGAGGAGCCCCCCGACGCGGCUCUUCUCCCGCCACCACG 351
Tshwane2             AGAACACAACCCGGUGAGAGGAGAGGAACCCCCCGGCGAGGCUCCUCUCCCACCCCCACG 352
Jozini               AGAAAACAAUCCAGUGAGAGGAGAGGAACGCCCCGGCGAGGCUCCUCUCCCACCCCCACG 351
Tshwane1             AGAAAACAACCCGGUGCGAGGAGAGGAACCCCCCGACGAGGCUCCUCUCCCACCACCACG 353
Cocoindia            AGAAAACAACCCGGUGCGAGAAGAGGAACCCCCCGACGAGGCUCUUCUCCCACCACCACC 351
Umbumbulu            AGAAAACAACCCGGUGGGAGGAGAGGAACCCCCCGACGAGGCUCCUCUCCCACCACCACG 352
Eshowe1              AGAAAACACCCCCGUGAGAGGAGAGGAACCCCCCGACGAGGCUCCUCUCCCCACCCCCACG 351
Mbazwana             AGAAAACACUCCCGGGGGAGGAGAGGAGCCCCCCGACGAGGCUCCUCUCCCGCCACCACG 352
Ntumeni              AGAAAACACUCCCGAGAGAGAAGAGGAGCCCCCCGACGCGGCUCCUCUCCCGCCACCACG 352
Hluhluwe             AGAAAACAAUCCGGAGAGAGGAGAGGAGCCCCCCGGCGAGGCUCCUCUCCCACCACCACG 352
Durban               AGAAAACACUCCCGAGAGAGAAGAGGACCCCCCCGGCGCGUCUCCUCUCCCGCCCCCACG 352
Willowvale           AGAAAACACUCCGGAGAGAGAAGAGGACCCCCCCGGCGAGUCUCCUCUCCCACCCCCACG 351
Isipingo             AGAAAACACUCCCGAGAGAGAAGAGGAGCCCCCCGACGCGGCUCUUCUACCGCCACCACG 352
Tshwane3             AGAAAACACUCCGGAGAGAGAAGAGGAGCCCCCCGGCGGGUCUCUUCCCCCGCCACCCCG 353
Maphumulo            AGAACACAACCCAGUCCGGGGAGAGGAACGGCCCACCGAGUCUGCUCUCCCACCGCCACC 352
Empangeni            AGAACACAACCCAGUCCGGGGAAAGGAACGGCCCAGCGAGUCUGCUCUCCCACCGCCACG 350
Ghana                CGAACAGAACCCAGUCCGGGGAGAGGAACGGCCCACCGAGGCUGCUCUCCCACCGCCACC 352
Mangozi              CGAAGAGAACCCAGUCCGGAGAGAGGAACGGCCCACCGAGGCAGCUCUCCGACCGCGACC 346
Makatini             CGAAGAGAACCCAGUCCGGAGAGAGGAACGGCCCACCGAGGCAGCUCUCCGACCGCGACC 347
MakatiniRes          CGAAGAGAACCCAGUCCGGAGAGAGGAACGGCCCACCGAGGCAGCUCUCCGACCGCGACC 347
Nigeria              CAAAAAGAACCCAAUCCGGAGAGAGGAAAGGACCGCCGAGGCGGCUCUCCUACCGCAACC 346
Nelspruit            CGAAGAGAACCCAAUCCGGGGAGAGGAACGGCCCACCGAGGCGGCUCUCCUACCGCAACC 346
                       ** * *  **     *   * ****     **  ** * *   **  *  ** *  *

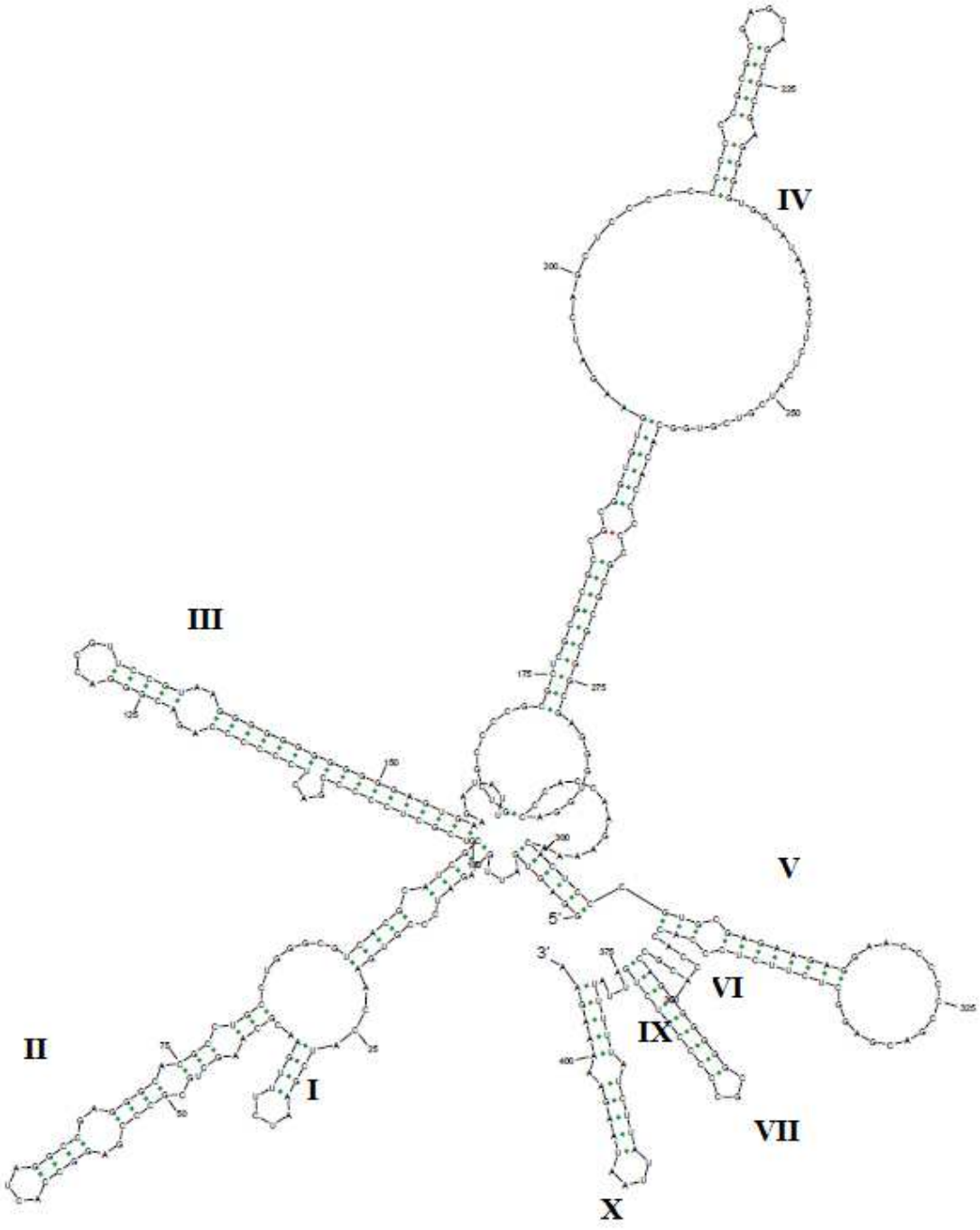
Lusikisiki           UCAGGUGAGGCGCCCCCCUCUGAUUAUUUUUAUCUUAUUAAUAAGAAGAAGAA----- 404
Eshowe2              GCAGGUGAGGCGCCCCCCCCUGAUUAUUUUUAUCUUAUUAAUAAGAGGAAGAA----- 405
Pietermaritzburg     CCAGGGGGGGCGCCCCCCUCUGAUUAUUUUUAUCUUAUUAAUAAGAAAAAGAA----- 405
Modderfontein        GCUGGUGGGGCGGCCCCCUCUGAUUAUUUUUAUCUUAUUAAUAAGAAGAAGAA----- 404
Tshwane2             UCAGGUGGGGCGGCCCCCUCUGUUUAUUUUUAUCAUAUUAAUAAGAAAAAGAA----- 405
Jozini               CCAGGUGAGGCGGCCCCCUCUGUUUAUUUUUAUCAUAUUAAUAAGAAAAAGAA----- 404
Tshwane1             CCAGGUGAGGCGGCCCCCUCUGUUUAUUUUUAUCUUAUUAAUAAGAAGAAGAA----- 406
Cocoindia            CCAGGUGAGGCGGCCCCCCCUGAUUAUUUUUAUCUUAUUAAUAAGAAGAAGAA----- 404
Umbumbulu            CCAGGUGGGGCGCCCCCCCCUGUUUAUUUUUAUCUUAUUAAUAAGAAGAAGAA----- 405
Eshowe1              UCAGGUGGGGCGGCCCCCUCUGUUUAUUUUUAUCUUAUUAAUAAGAAAAAGAA----- 404
Mbazwana             UCUGGUGGGGCGGCCCCCUCUGAUUAUUUUUAUCUUAUUAAUAAGAAAAAGAA----- 405
Ntumeni              UCAGGUGGGGCGCCCCCCUCUGAUUAUUUUUAUCUUAAUAAGAAGAAAAAGAA----- 405
Hluhluwe             UCAGGUGGGGCGCCCCCCUCUGUUUAUUUUUAUCUUUUUAAGAAGAAAAAGAA----- 405
Durban               UCAGGGGGGGCCCCCCCCUCUGUUUAUUUUUAUCUUUAUAAGGAGAAAAAGAA----- 405
Willowvale           UCAGGUGGGGCCGCCCCCUCUGUUUAUUUUUAUCUUUAUAAGAAGAAAAAGAA----- 404
Isipingo             UGAGGUGGGGCGCCCCCCUCUGAUUAUAUUUAUCUUAAUAAGCAGAAAAAGAA----- 405
Tshwane3             UGAGGCGGGGCGCCCCCCUCUGAUUAUAUUUAUCUUUAUAAGAAGAAAAAGAA----- 406
Maphumulo            CCAGGUCAGGCCGCCCCACCCGUUGAAUUUUAUCAUAUCAAUAAGCAAAGGAA----- 405
Empangeni            CCAGGUCAGGCCGCCCCACCCGUUGAAUUUUAUCAUAUCAAUAAGCAAAGGAA----- 403
Ghana                CCAGGUCAGGCCGCCCCACCCGUUGAAUUUUAUCAUUUCAAUAAGGAAAGGAA----- 405
Mangozi              CCAGGUCAGGCGGGGCCACCCGCUGAGUUUAAGCAUAUCAAUAAGCGGAGGAA----- 399
Makatini             CCAGGUCAGGCGGGGCCACCCGCUGAGUUUAAGCAUAUCAAUAAGCGGAGGAA----- 400
MakatiniRes          CCAGGUCAGGCGGGGCCACCCGCUGAGUUUAAGCAUAUCAAUAAGCGGAGGAAA---- 401
Nigeria              CCAGGUCAGGCGGGGCCACCCCCUGAGUUUAAUCAUAUCAUUAAACGGAGGAAAGGAA 404
Nelspruit            CCAGGUCAGGCGGGGCCACCCGCUGAGUUUAGUU-UAUCAAUAAGCGAAGGAAAGGAA 403
                        **   ***    **  *   * *  **     *   *   *    * ***
```

ANNEXURE B: Predicted secondary structures of the 25 accessions of taro



PIETERMARITZBURG

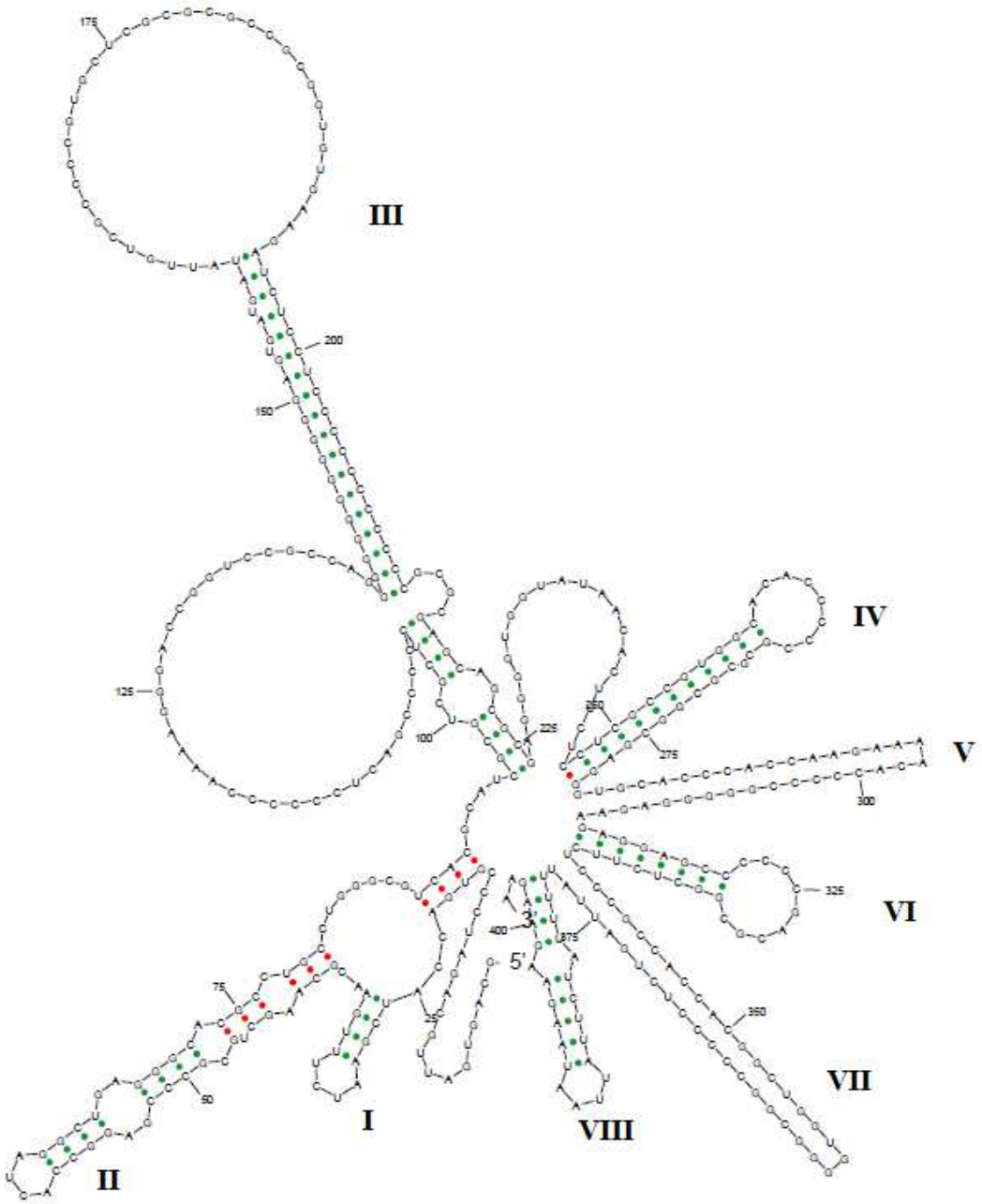
Ensemble Centroid



ΔG°_{37} = -165.76

MODDERFONTEIN

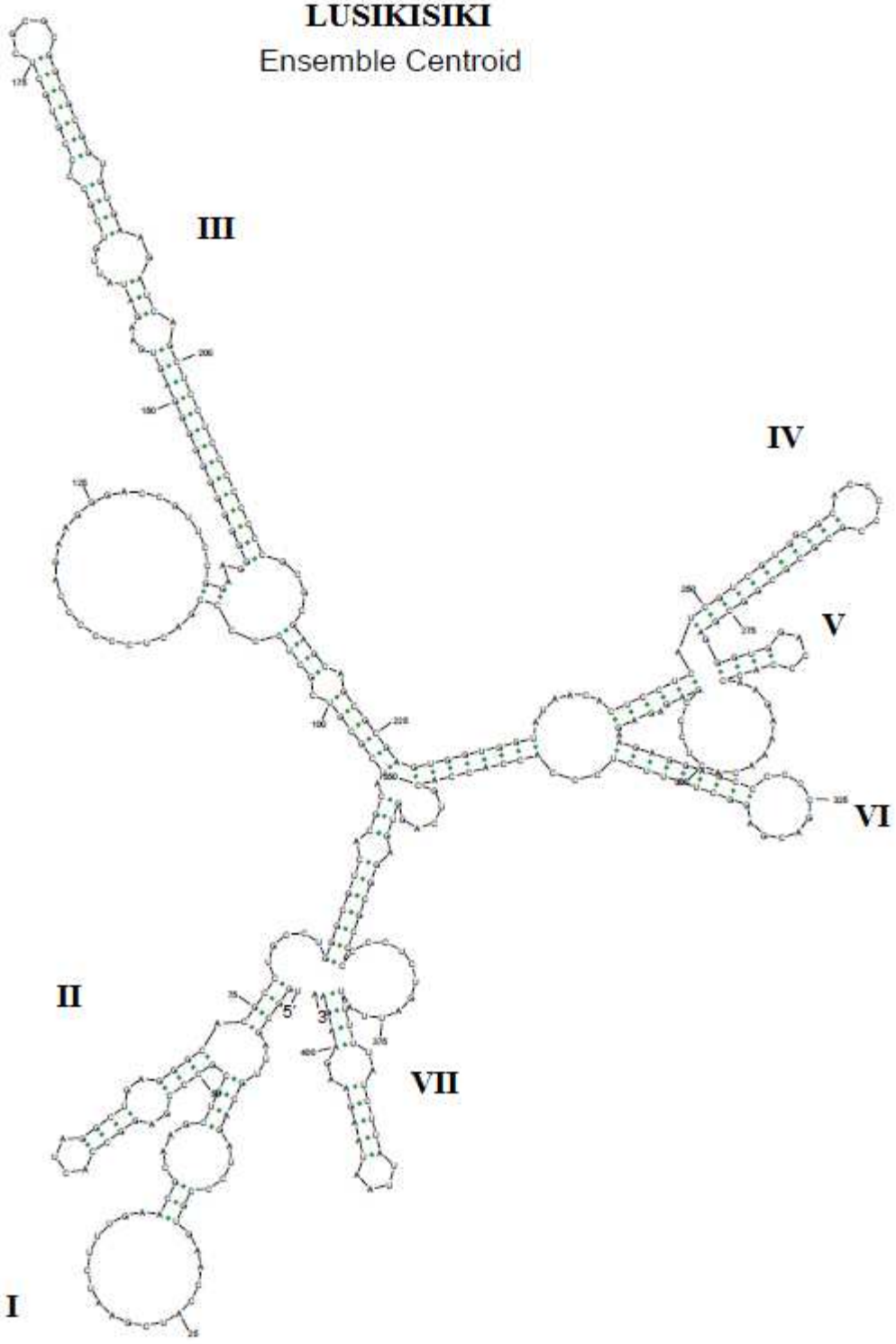
Ensemble Centroid



ΔG°_{37} = -110.88

LUSIKISIKI

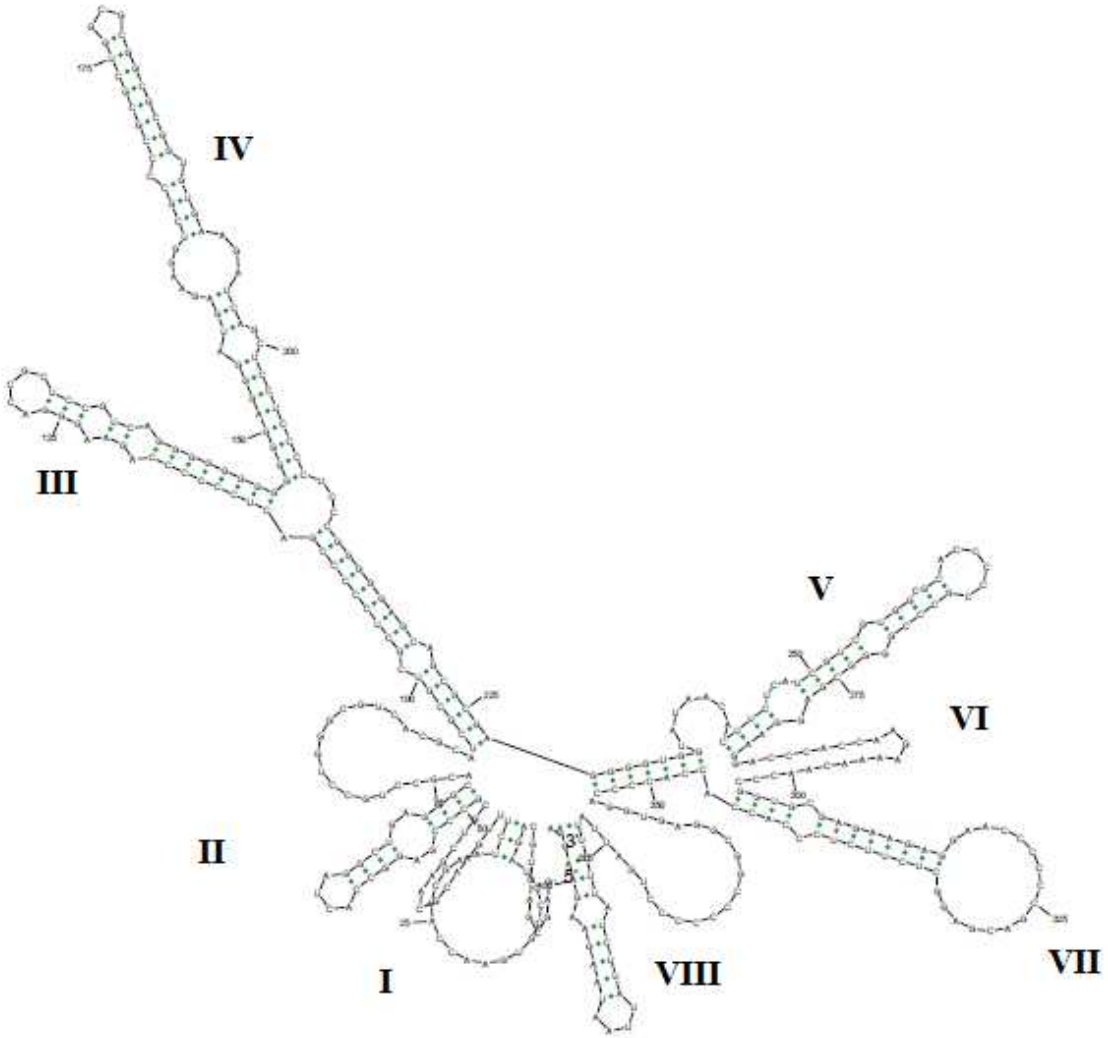
Ensemble Centroid



ΔG°_{37} = -175.80

COCOINDIA

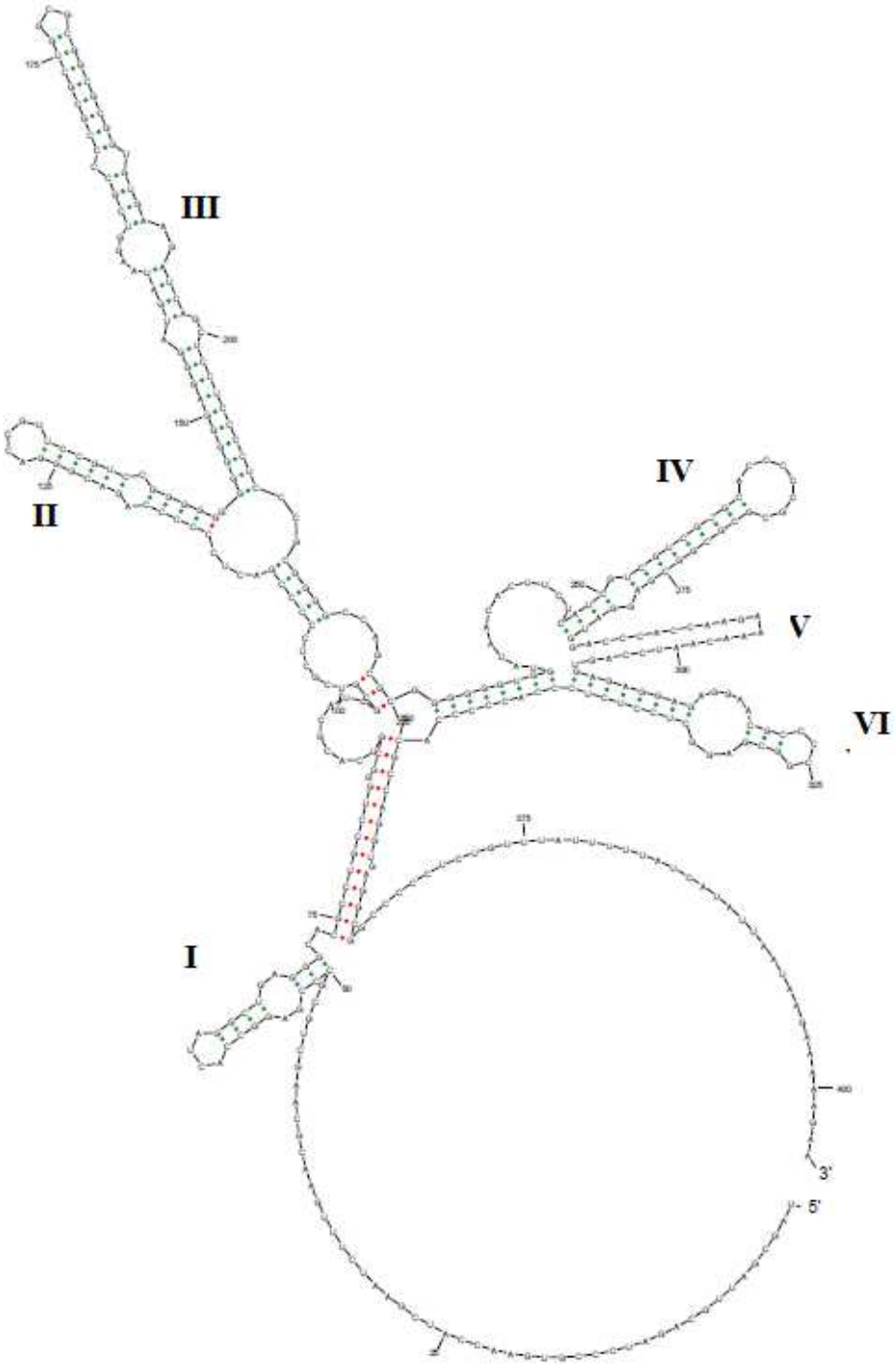
Ensemble Centroid



ΔG°_{37} = -162.20

JOZINI

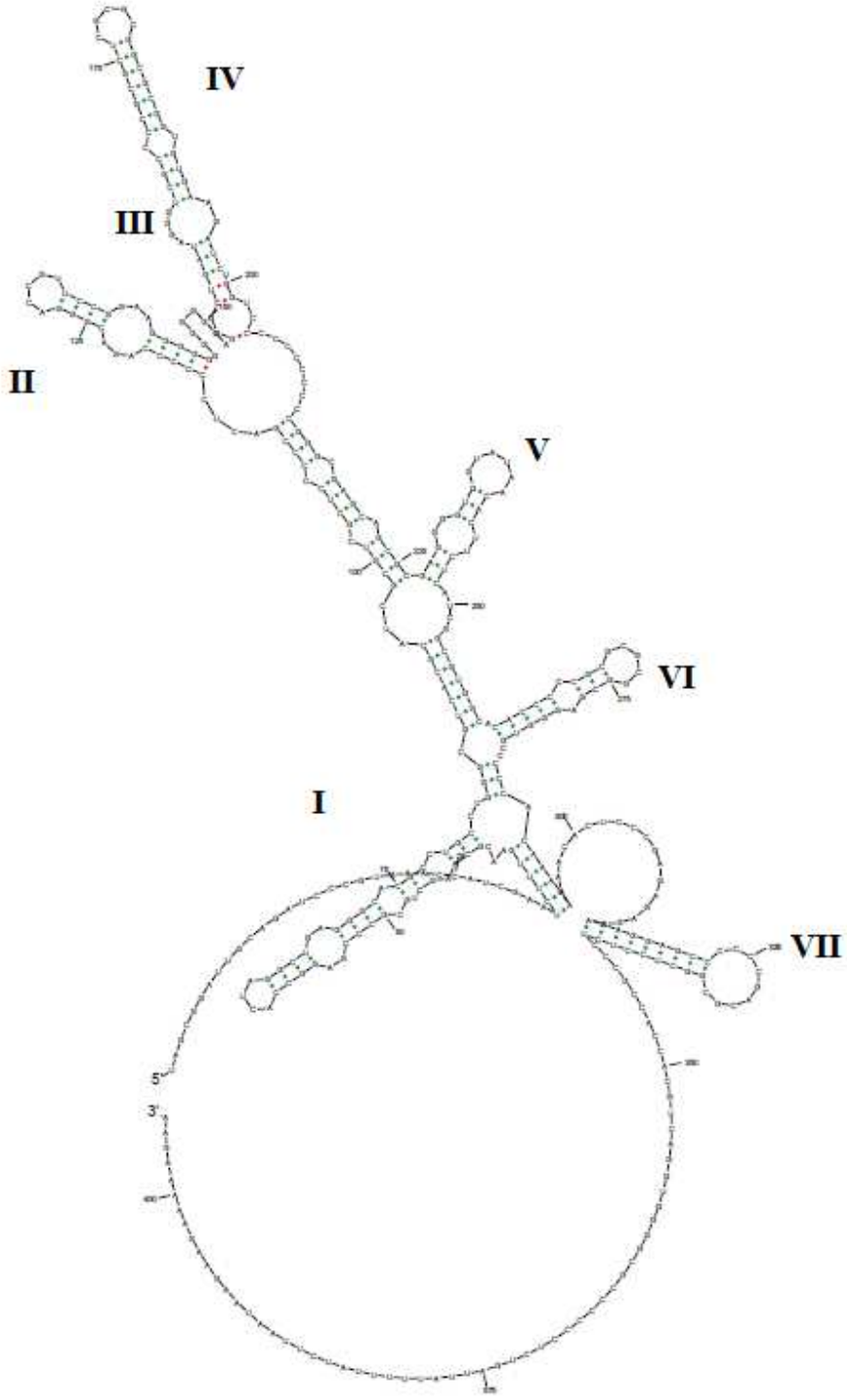
Ensemble Centroid



ΔG°_{37} = -173.70

NTUMENI

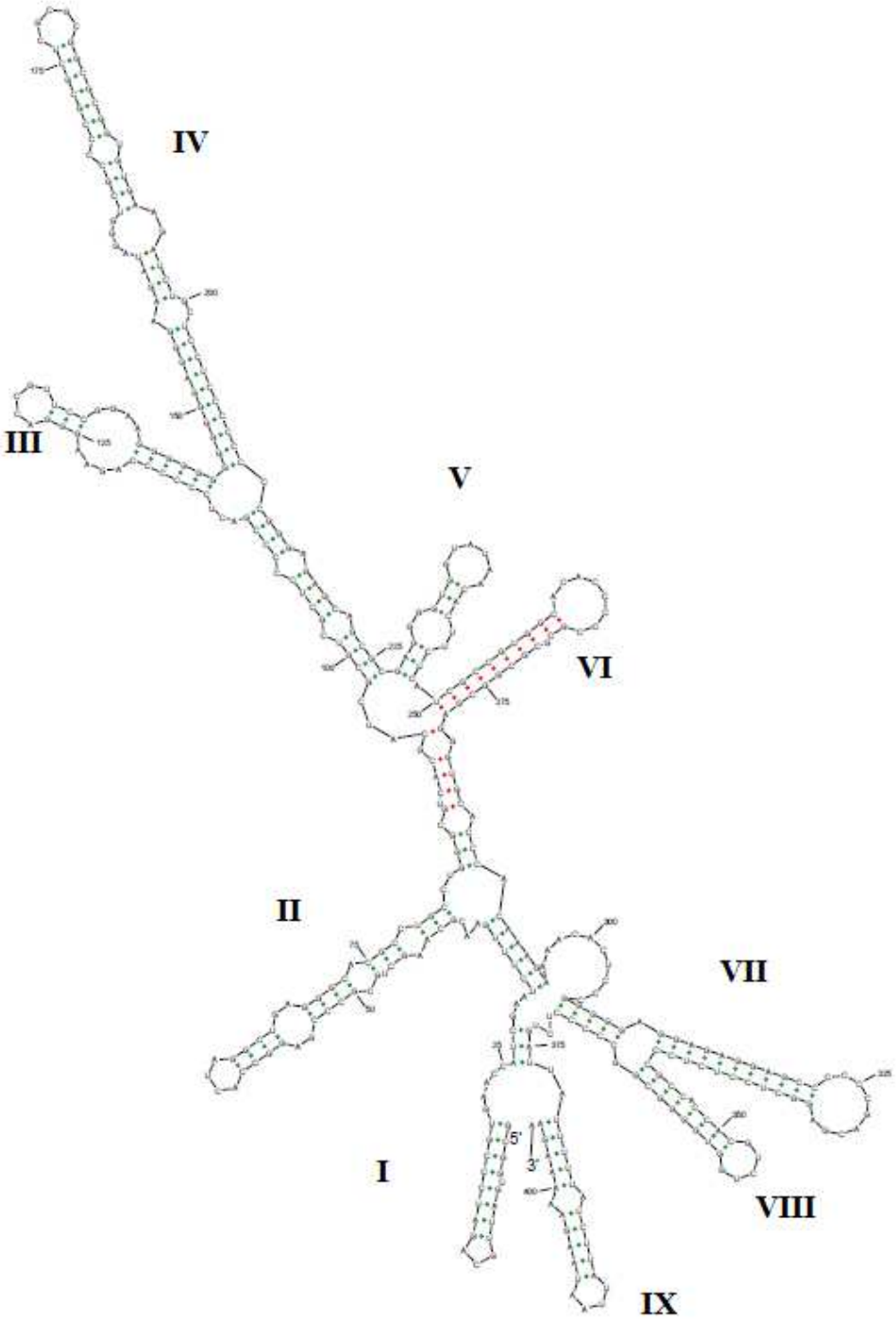
Ensemble Centroid



ΔG°_{37} = -145.20

MBAZWANA

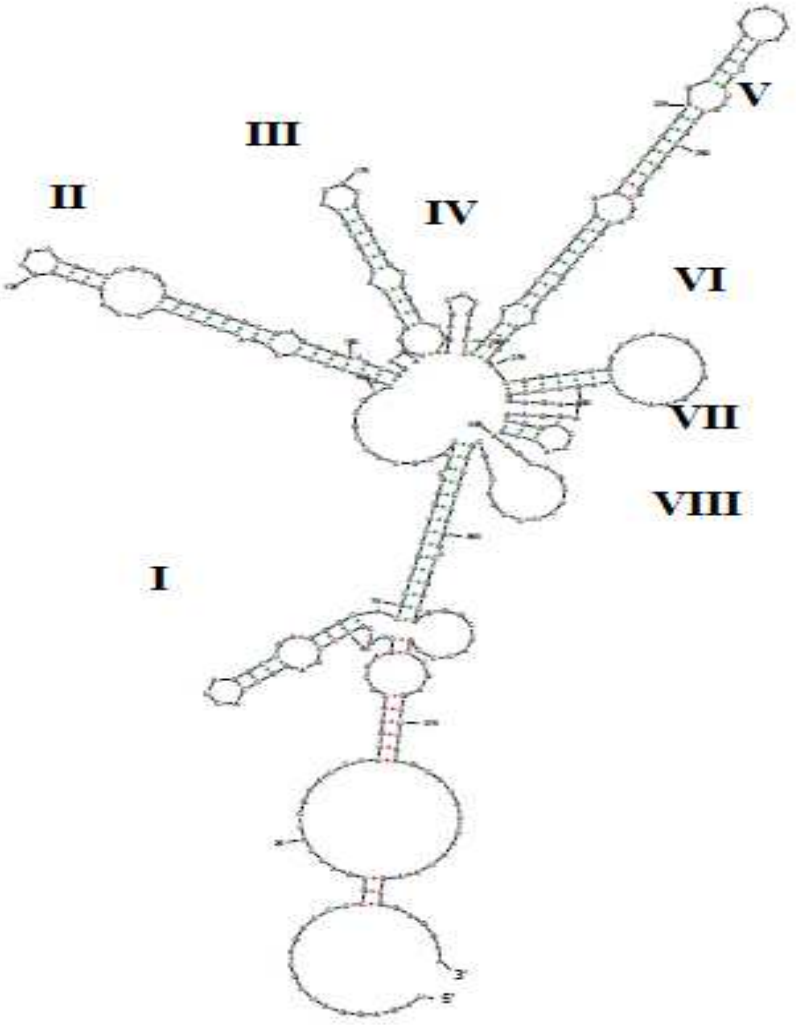
Ensemble Centroid



ΔG°_{37} = -206.80

MANGOZI

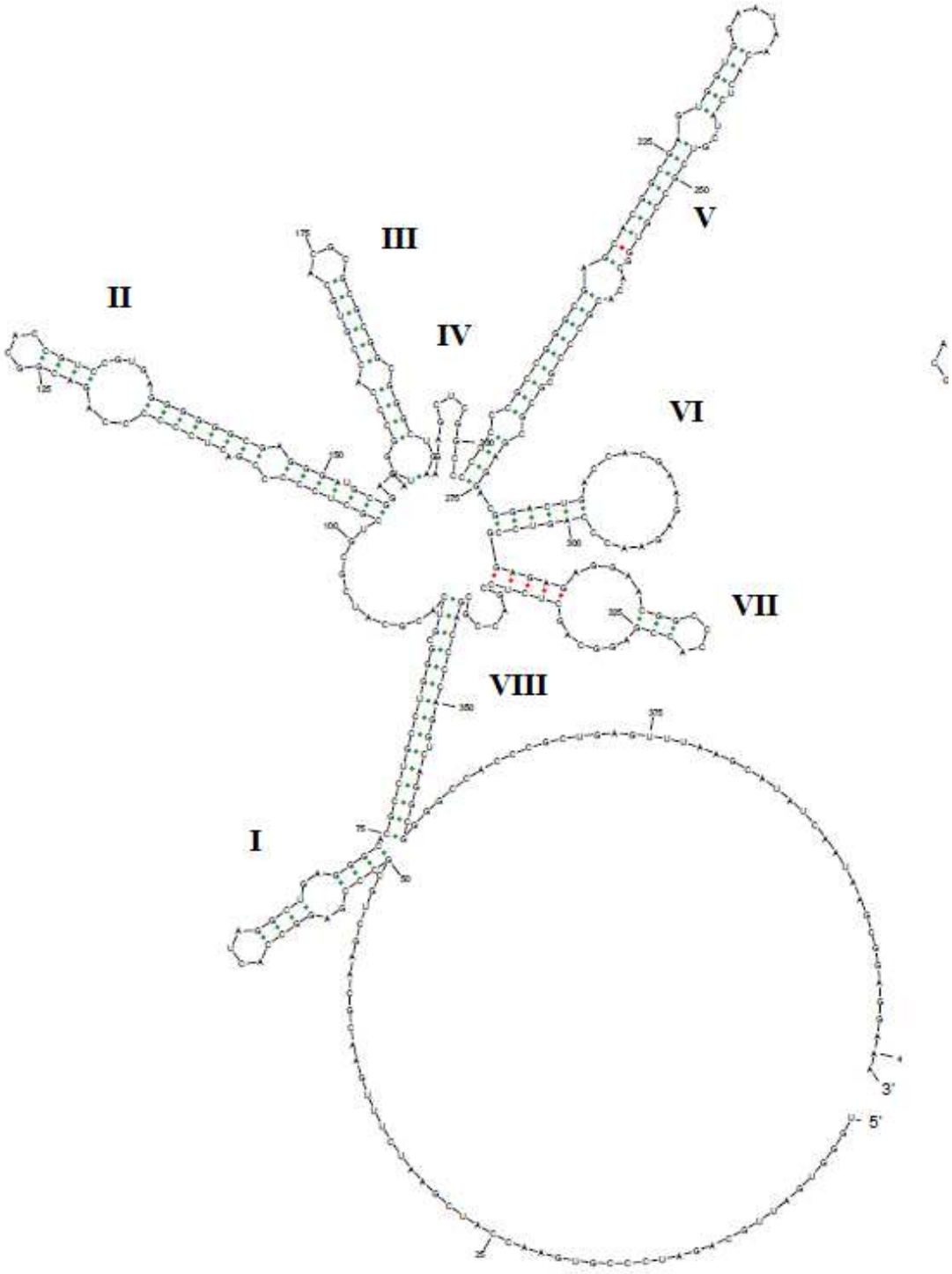
Ensemble Centroid



ΔG°_{ens} = -162.50

MAKATINI RES

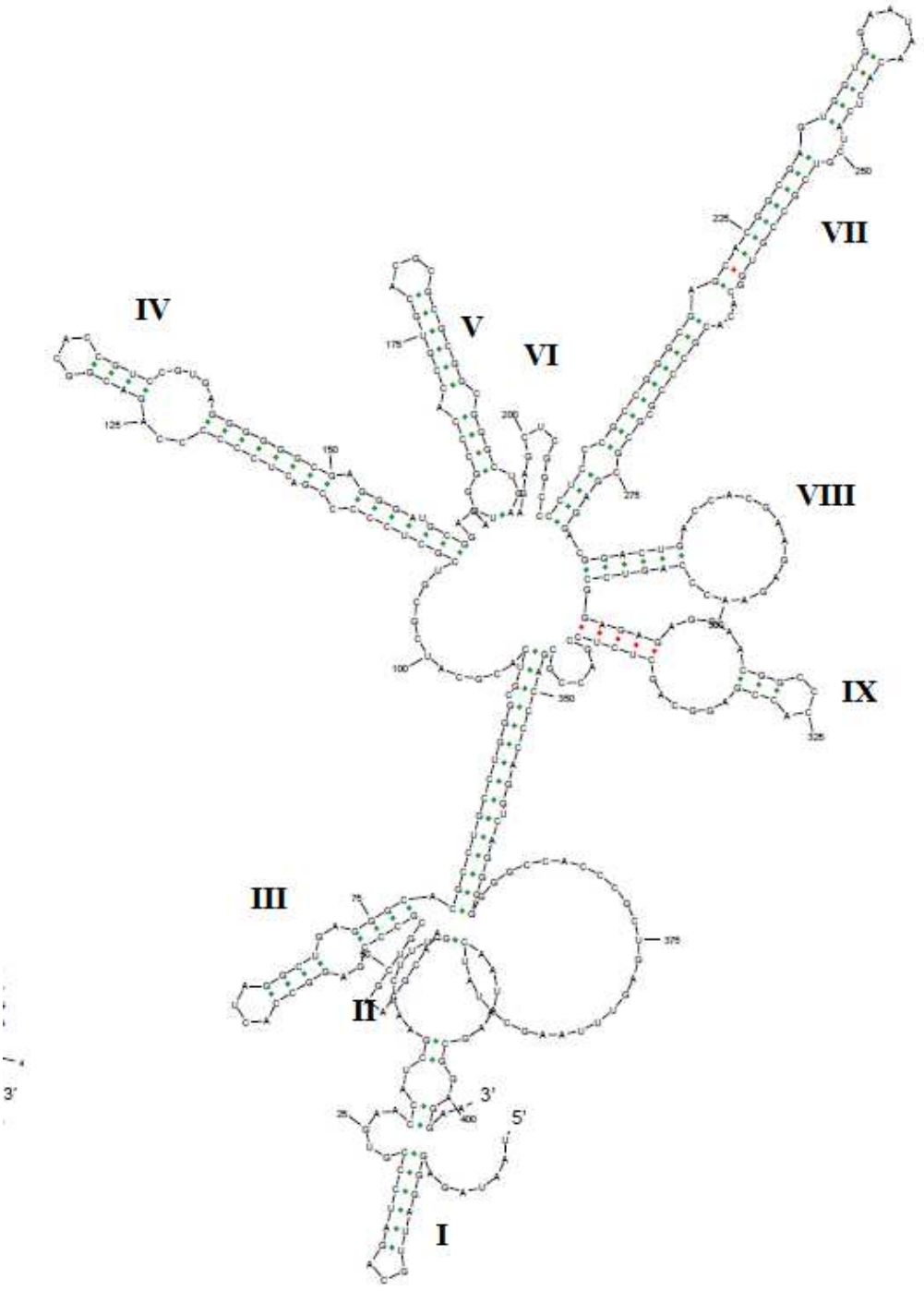
Ensemble Centroid



ΔG°_{37} = -159.80

MAKATINI

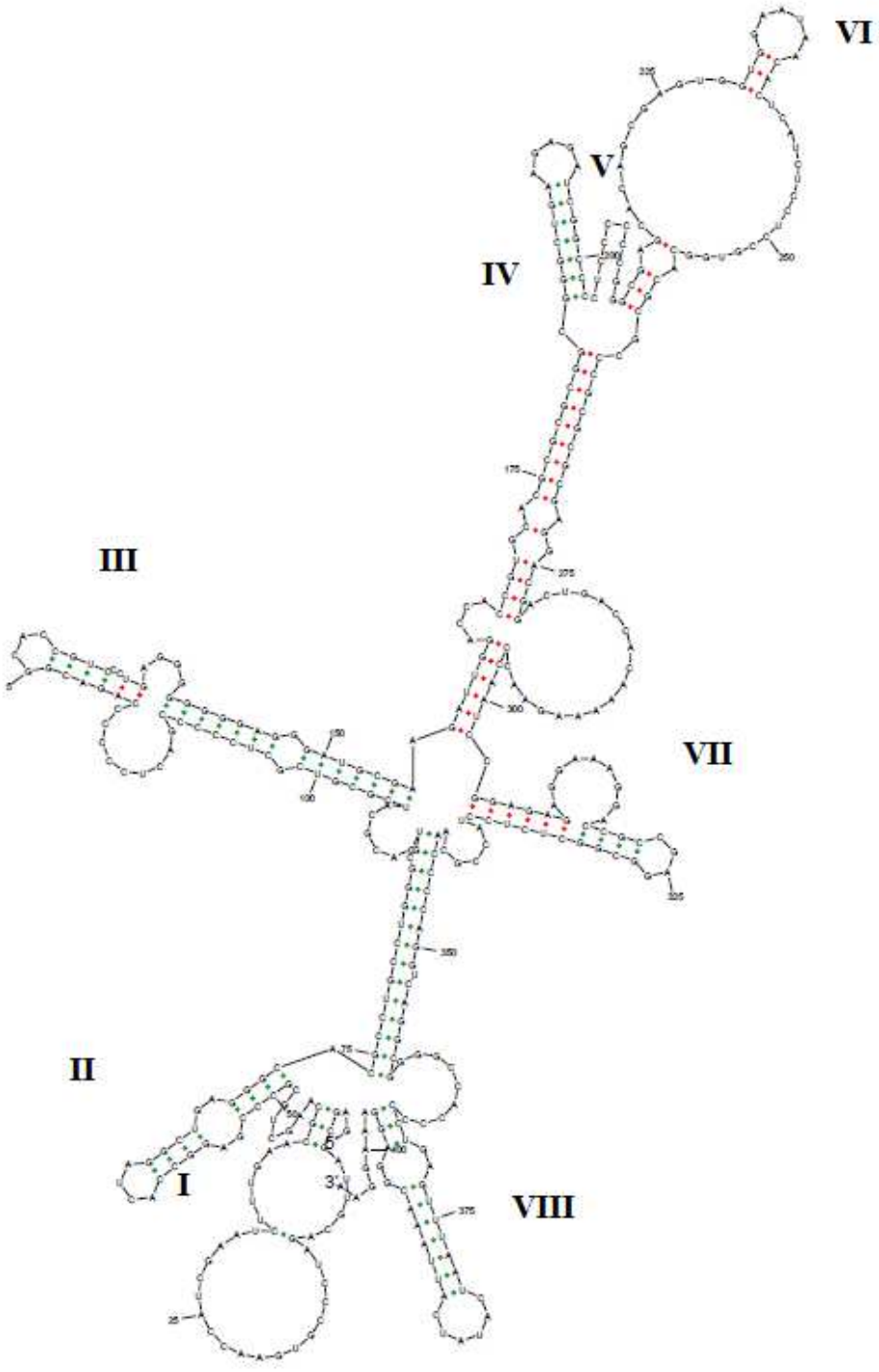
Ensemble Centroid



ΔG°_{str} = -169.10

NIGERIA

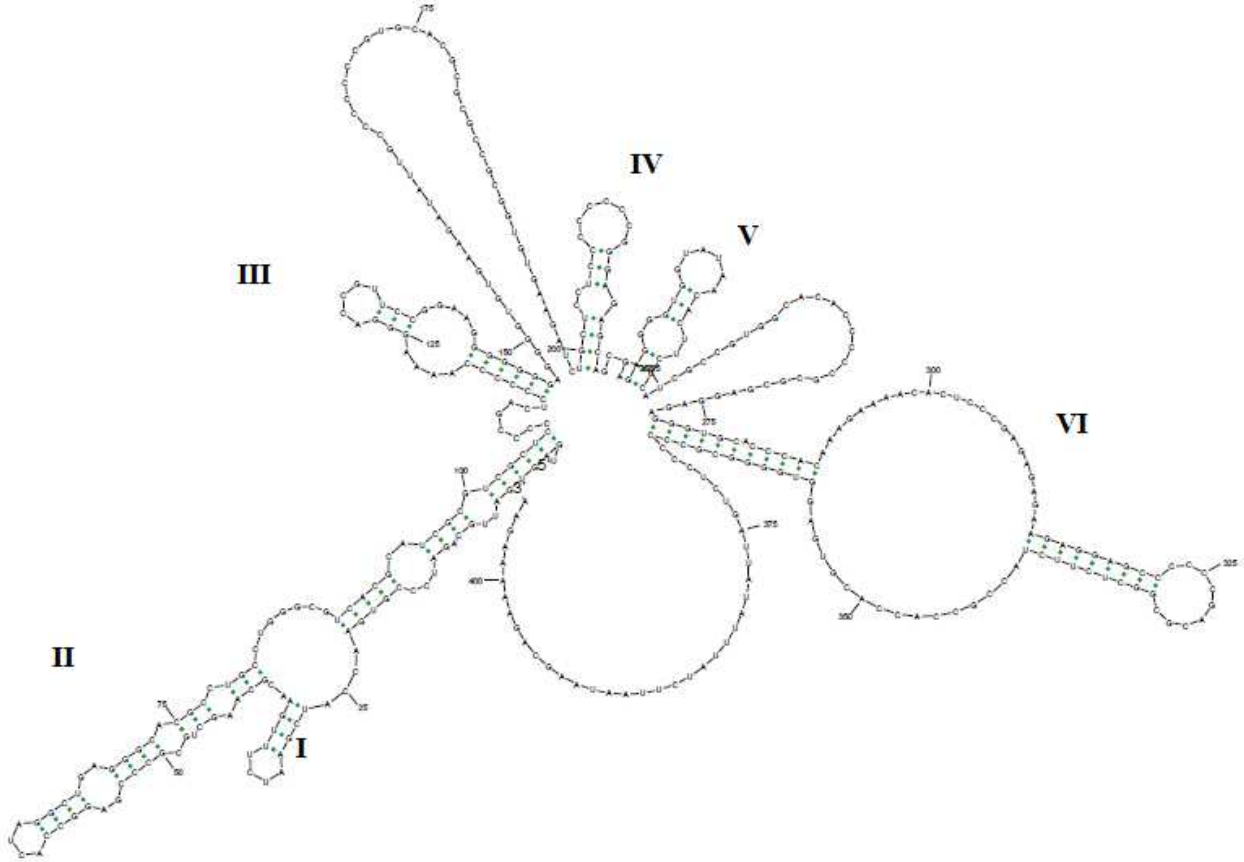
Ensemble Centroid



ΔG°_{37} = -142.40

ISIPINGO

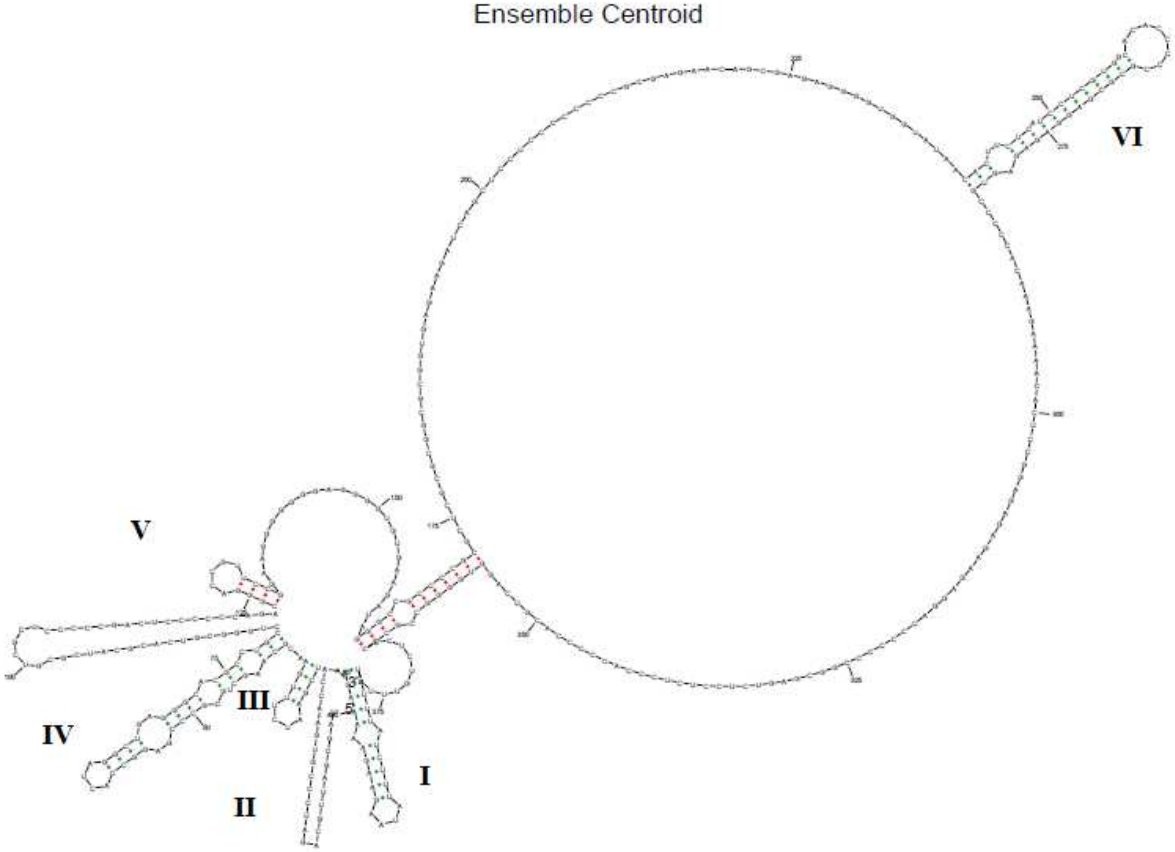
Ensemble Centroid



ΔG°_{37} = -103.52

WILLOWVALE

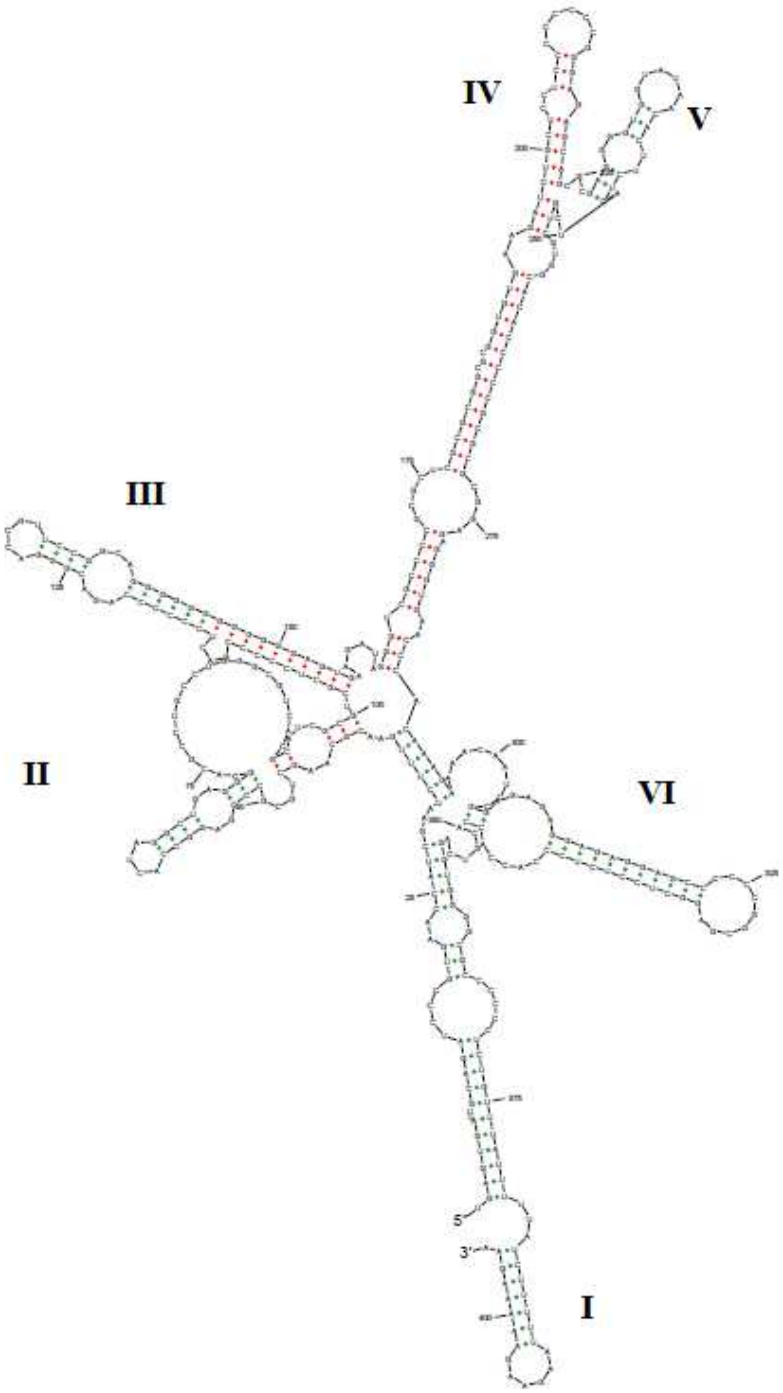
Ensemble Centroid



ΔG°_{ss} = -67.64

HLUHLUWE

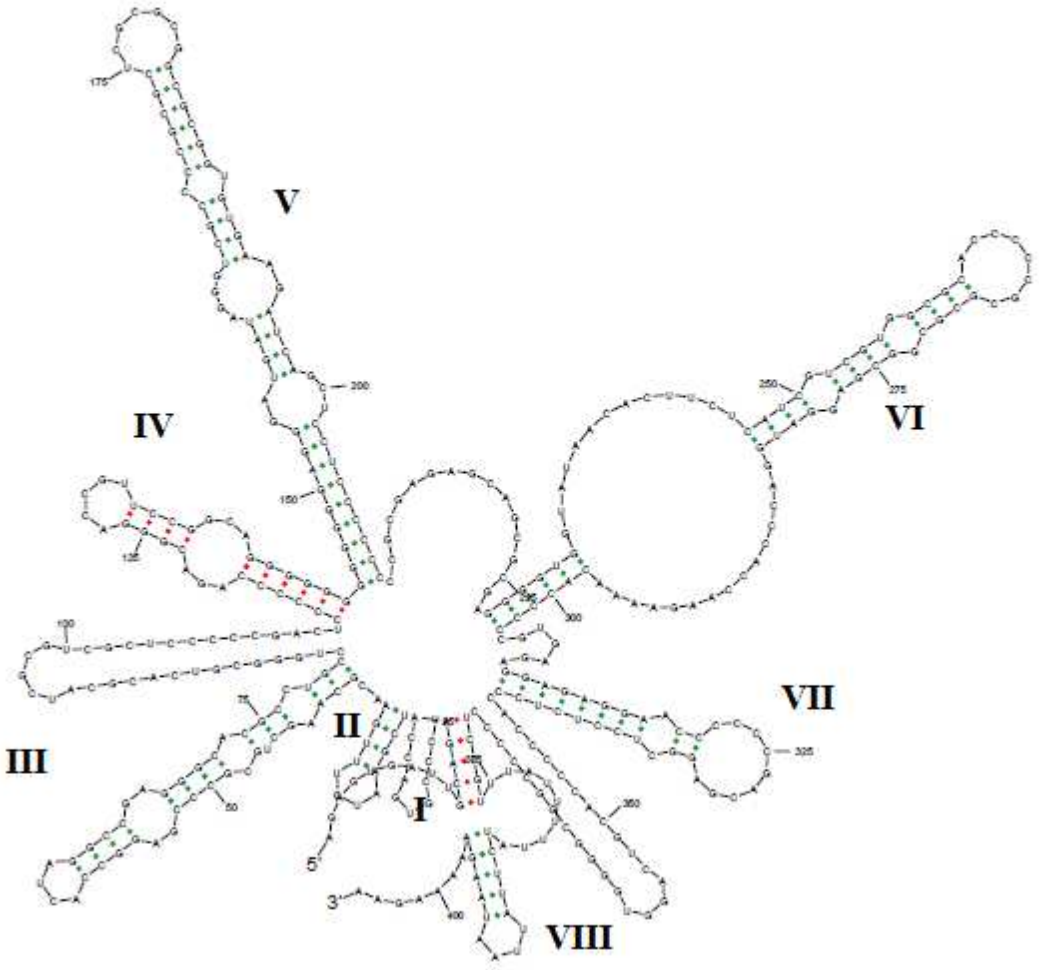
Ensemble Centroid



$\Delta G^{\circ}_{37} = -163.10$

ESHOWE 1

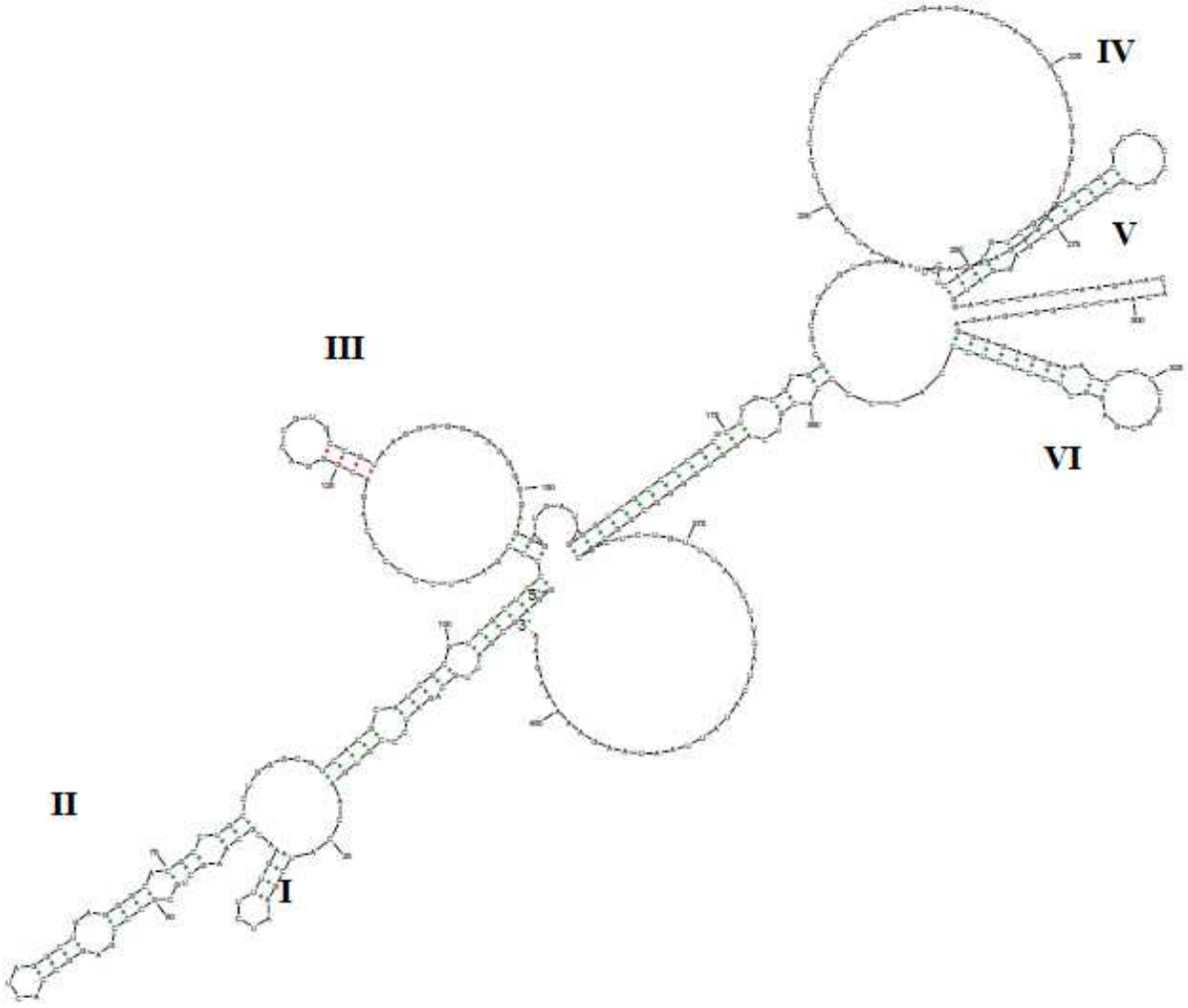
Ensemble Centroid



ΔG°_{ens} = -140.30

TSHWANE 2

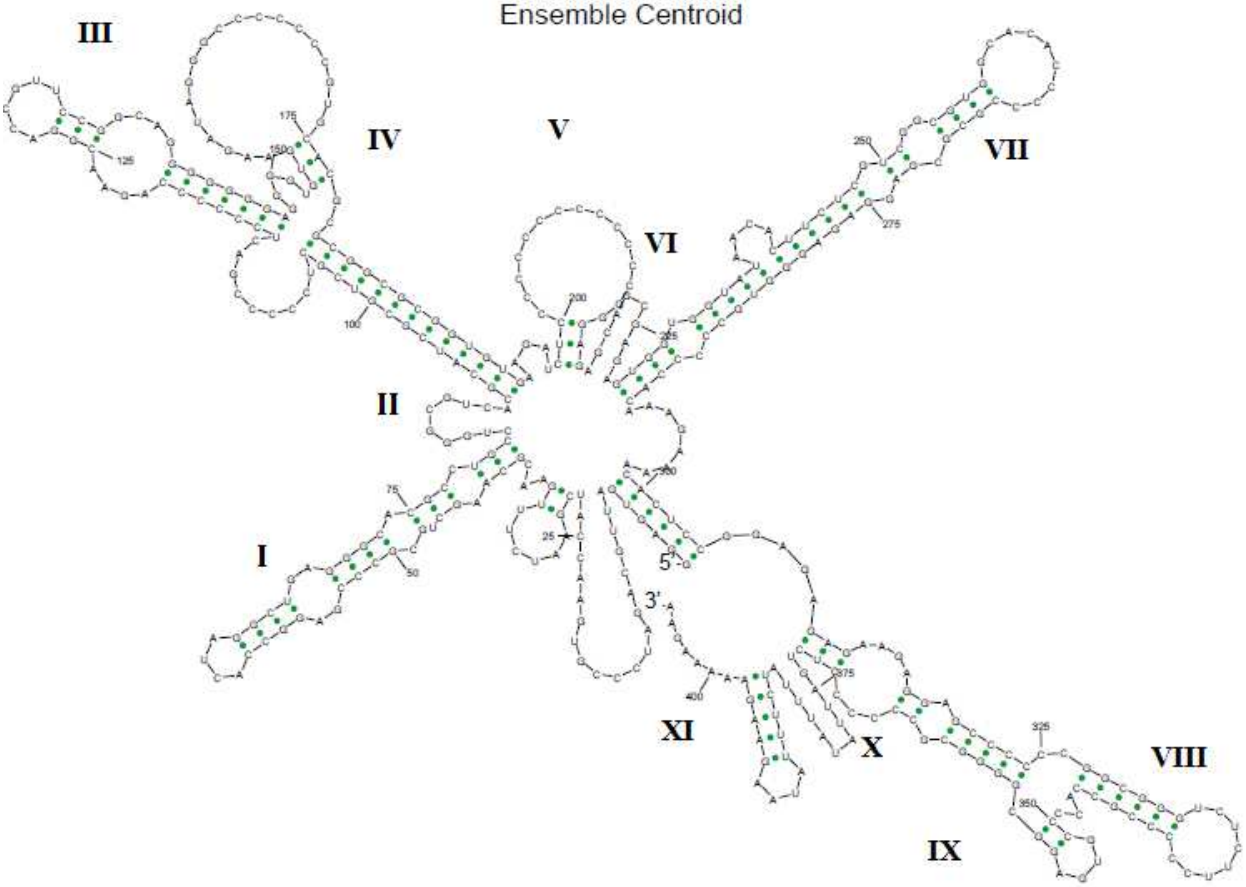
Ensemble Centroid



ΔG°_{37} = -115.87

TSHWANE 3

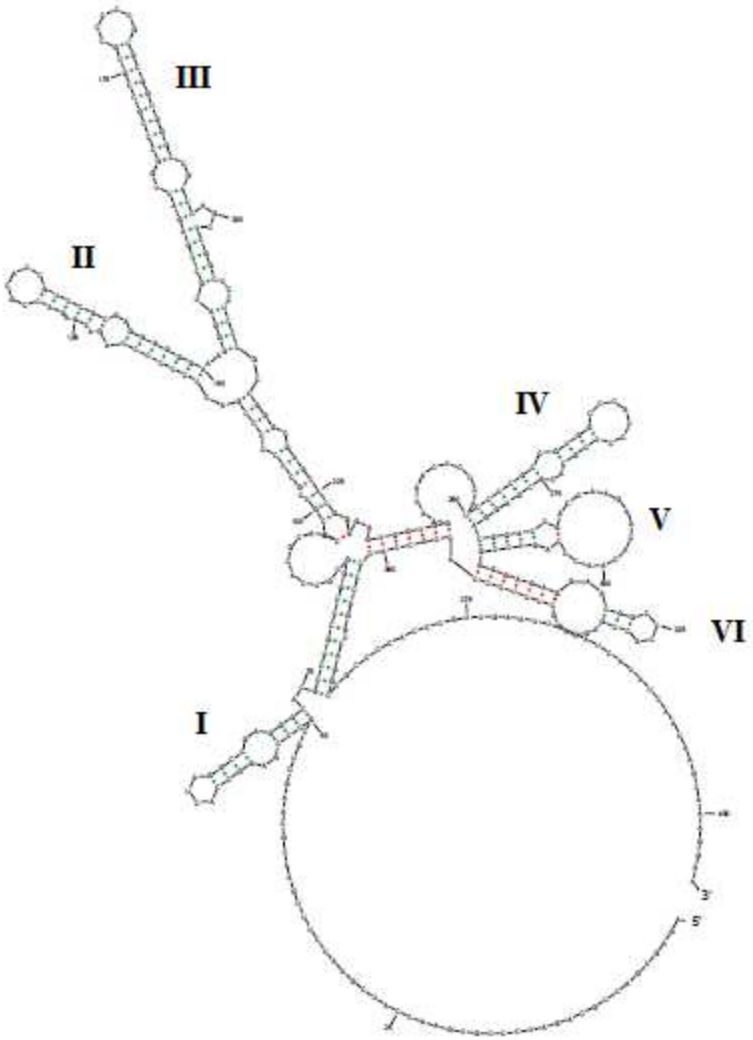
Ensemble Centroid



ΔG°_{37} = -138.40

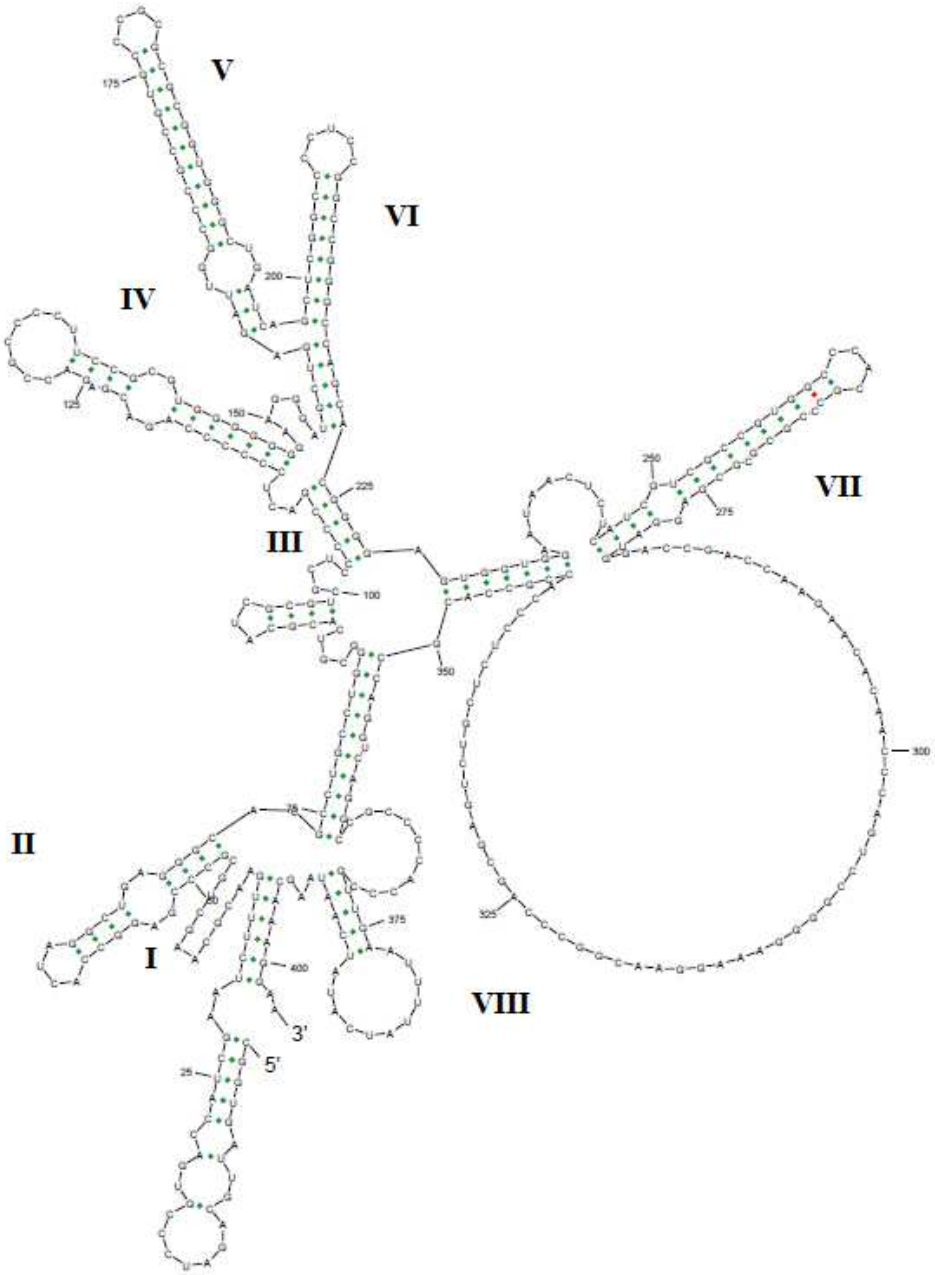
Maphumulo

Ensemble Centroid



ΔG° = -165.60

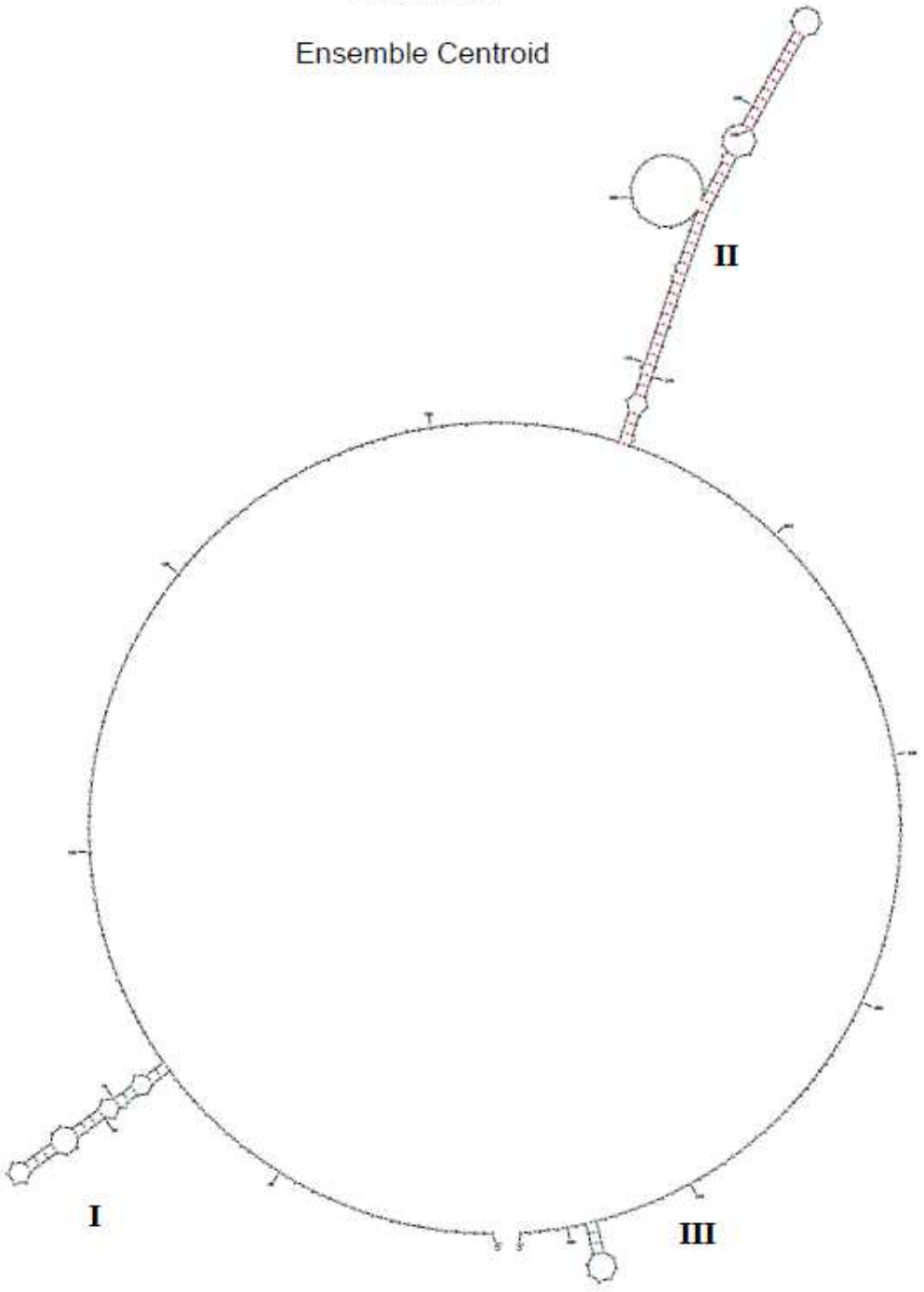
EMPANGENI
Ensemble Centroid



ΔG°_{37} = -157.09

DURBAN

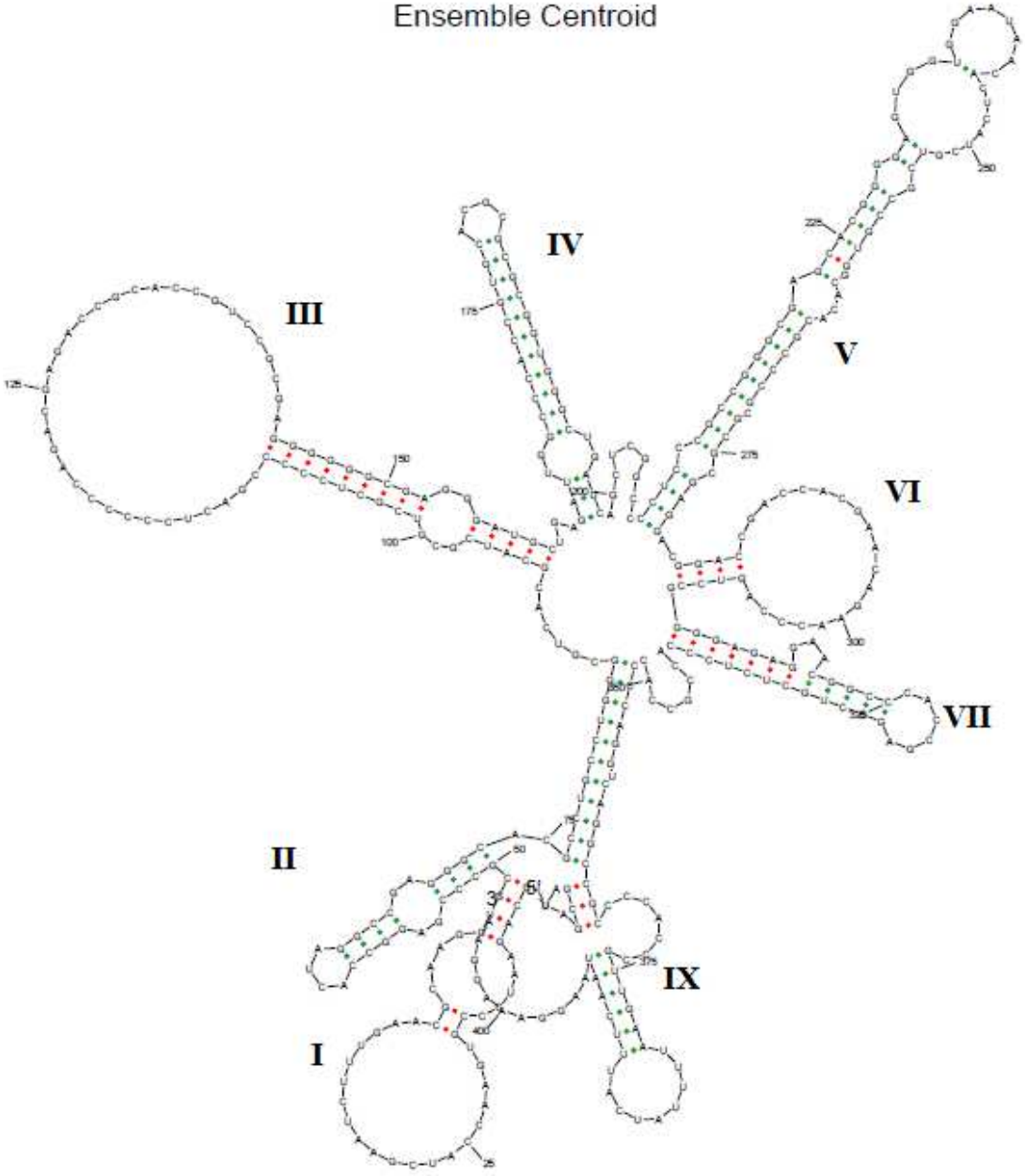
Ensemble Centroid



ΔG°_{37} = -83.70

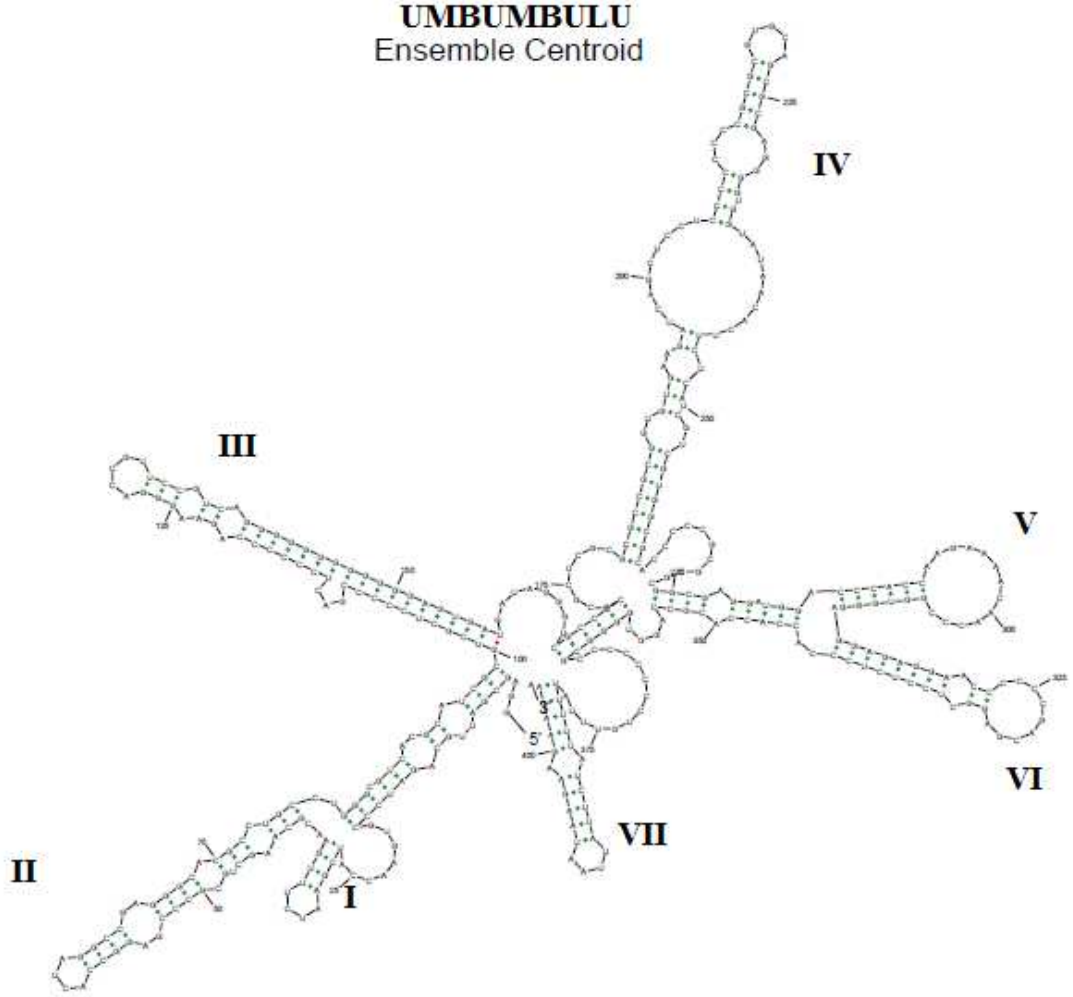
GHANA

Ensemble Centroid

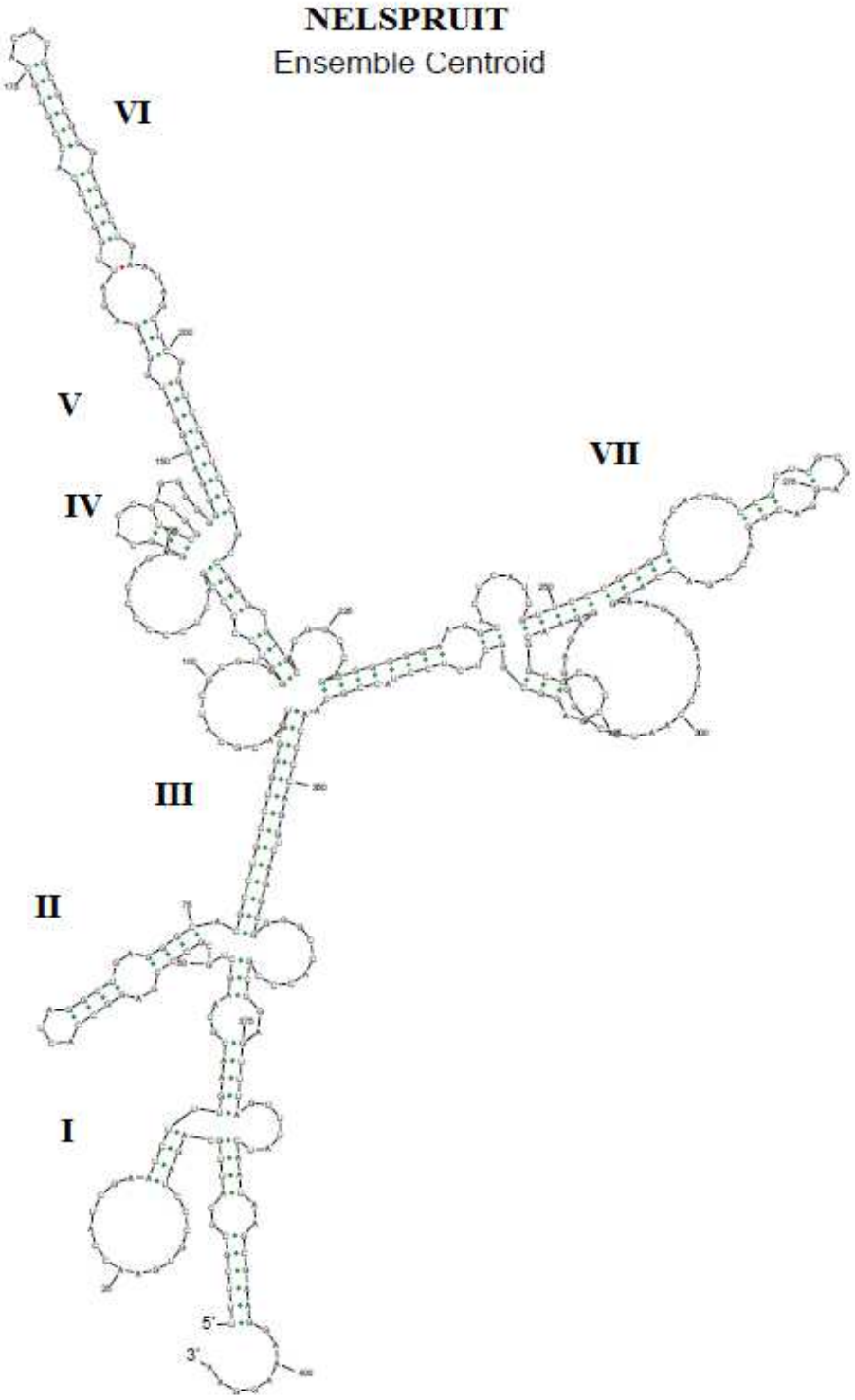


ΔG°_{37} = -154.76

UMBUMBULU
Ensemble Centroid



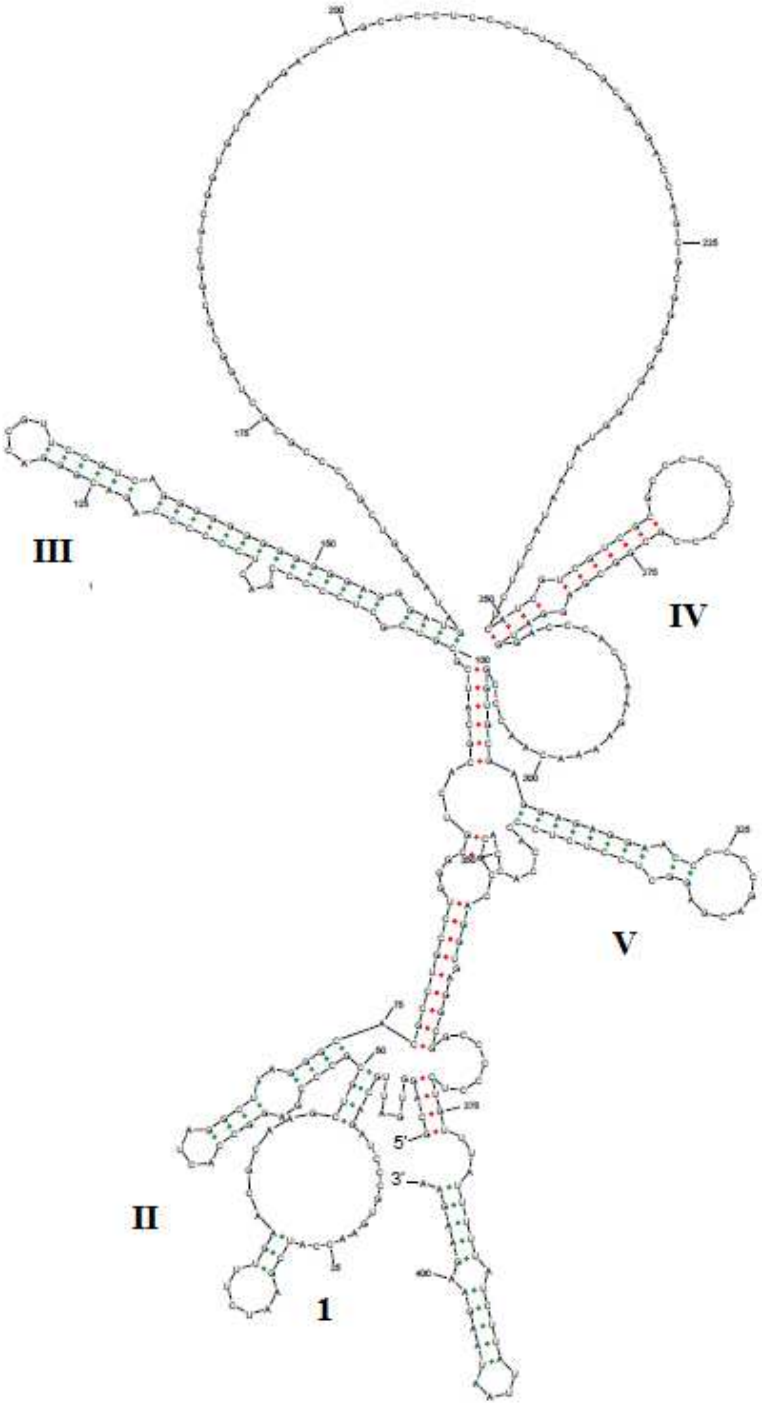
ΔG°_{37} = -166.80



ΔG°_{37} = -156.40

TSHWANE 1

Ensemble Centroid



ΔG°_{37} = -128.30