MORPHOLOGICAL AND MOLECULAR DIVERSITY OF STINGING NETTLE (*Urtica simensis*) FROM NORTHERN ETHIOPIA

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ABSTRACT

Urtica simensis is an erect perennial herb among the species of nettle which belongs to the family *Urticaceae* that is endemic to Ethiopia and locally known as Samma. It is cooked and consumed as vegetable in some parts of Ethiopia. It has medicinal properties where traditionally people use it in treating different types of diseases, including infectious diseases like allergies, diabetes and Malaria. This study was conducted to evaluate the diversity of *Urtica simensis* to address and fill the gap on its genetic variability and distribution using morphological characters and ISSR DNA markers. A total 133 plant samples were collected from Northern growing areas of Ethiopia (Gondar and Mekelle), based on variations in morphological characters. Morphological characterization was conducted on six morphological traits which demonstrated variation based on PCA and correlation matrix analysis. Molecular characterization was carried out using ISSR markers where, only 5 were selected out of 16 ISSR primers that produced a total of 445 scorable bands and 30 polymorphic loci, 100% of polymorphic bands, 1.88 genetic diversity and 2.75 Shannon index from a collection of 133 plant samples. Highest genetic diversity (H) was found to be 0.4286 and Shannon Information index (I) was 0.6197. The highest genetic diversity was indicated in plant samples from Mekelle compared to samples from Gondar (0.3462 and 0.3152 respectively). The Dendrogram based on Jaccard's similarity coefficients generated by UPGMA cluster analysis using morphological and ISSR data shows major and minor clusters with broad distribution of *Urtica simensis* individuals over the entire tree which indicates the low divergence in morphological appearance among populations from both study areas. Based on the results of this study, morphological and ISSR markers were effective in studying genetic diversity of *Urtica simensis* demonstrated by variations in terms of morphological appearances and genetic variability. These results

have valuable effect on characterization of *Urtica simensis* genetic resources in different parts of Ethiopia for conservation purposes.

DECLARATION

I, **BENAT EUSMAN ABDULKADIR**, do hereby declare to the senate of the Sokoine University of Agriculture that this dissertation is my original work, done within the period of registration and that it has neither been submitted nor been concurrently submitted for a higher degree award in any other institution.

_____ _____

Benat Eusman Abdulkadir Date

(MSc. Candidate)

The above declaration is confirmed by;

_____ _____

Prof. Paul Mbogo Kusolwa Date

(Supervisor)

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LIST OF ABBREVIATIONS AND SYMBOLS

AFLP	Amplified Fragment Length Polymorphism
AMOVA	Analysis of molecular variance
cm	centimeters
COA	College of Agriculture
DCSH	Department of Crop Science and Horticulture
DNA	Deoxyribo Nucleic Acids
ISSR	Inter-Simple Sequence Repeat
IUCEA	Inter-University Council for East Africa
km	kilometer
mm	millimeter
NTSYS	Numerical Taxonomic System
^{o}C	degree Celsius
PCR	Polymerase Chain Reaction
POPGENE	Population Genetic Analysis
RAPD	Random Amplified Polymorphic DNA
RFLPs	Restriction Fragment Length Polymorphisms
SACIDS	Southern African Center for Infectious Disease Surveillance
SNPs	Single Nucleotide Polymorphisms
SSRs	Simple Sequence Repeats
SUA	Sokoine University of Agriculture
UPGMA	Unweighted Pair Group Method of Arithmetic means
$^{\circ}$F	degree Fahrenheit
µl	microliter

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background and Justification of the Study

Stinging nettle belongs to the family *Urticaceae* and it is one of the widely available wild plants in temperate regions of the world. It is a representative of 30-45 species of common wildflowers that belong to the genus *Urtica*. Different species of the plant occur as a perennial plant in temperate regions of Asia, America, and Europe and it commonly grows in rich soils in forest clearings, old fields, and wasted places (Mamta and Preeti, 2014).

Urtica simensis is among the species of nettle that is endemic to Ethiopia locally known as Samma (Amharic). It is widely available in the wild and grows around the highlands of Ethiopia, specifically in the North and South Gondar, North and South Wello, North Shewa, Wag Hamra, Tigray region, highland of Sidama zone in Southern region and Arsi zone of Oromia region at 1500-3500 meters above sea level (Erenso and Maryo, 2014; Dereje *et al.*, 2016).

It is mostly found in grassland areas common in disturbed localities, often plentiful near houses and can be harvested whenever there is a need (Assefa *et al.*, 2013; Gebrezgabiher *et al.*, 2013; Alemayehu *et al.*, 2015). *Urtica simensis* (Samma) is dark green perennial wild species of plant predominantly leaves and young shoot parts collected by women and children usually used as an emergency famine food during a food shortage. It has great potential and contribution to food security to meet the nutritional demand of humans. The leaves and young shoots are traditionally cooked and consumed as a vegetable in some places of the country since they have high nutritive contents and grows throughout the year and available on-demand in nearby areas. It is one of the nontoxic and

well known locally accessible protein feed resources which contains all the essential amino acids. Its crude protein content is bounded from 25.1 to 26.3% besides it contains iron, calcium, phosphorus, potassium, sulfur, magnesium and it is also rich in vitamins A, C, K D, and B and up to 20% mineral salts, mainly salts of calcium, potassium, silicon, and nitrates (Friis, 1989; Assefa *et al.*, 2013; Dereje *et al.*, 2016; Keflie *et al.*, 2017) as shown in (Table 1).

Table 1: Some of nutrition, vitamin and mineral compositions of *Urtica simensis*

Compositions	Specific Content	Total value range	Reference
Nutrition	Crude Protein (CP)	25.1- 26.3 %	Assefa *et al*., 2013
Vitamins	Ascorbic Acid (C)	82.65 – 86.6 mg/100gm	Assefa *et al*., 2013
	Thiamine (B)	62.19 mg/100gm	Keflie *et al.*, 2017
Minerals	Iron (Fe)	38.4 – 47.0 mg/100gm	Assefa *et al*., 2013
	Calcium (Ca)	76..8 – 79.3 mg/100gm	Assefa *et al*., 2013
	Zinc (Zn)	2.87 – 5.80 mg/100gm	Assefa *et al*., 2013
	Potassium (K)	899.90 mg/100gm	Keflie *et al.*, 2017

Figure 1: Photograph of *Urtica simensis* captured during field survey in both study areas

It has medicinal properties and it is effective in the treatment of diseases. Traditionally people use a different part of the plant (leaves and root) in different forms for the treatment of ailments such as gonorrhea, allergies, blood pressure, gastritis, diarrhea, cough, and

other problems (Enyew *et al*., 2014; Alemayehu *et al*., 2015; kefalew *et al*., 2015; Maryo *et al*., 2015) and it is also used for the treatment of diabetes, malaria and peptic ulcer disease (Tsegaye *et al.*, 2009; wubetu *et al.*, 2017). Considering its plenty of uses mean its potential contribution to food security, nutrition, and health, become an alarming and need attention to evaluate its diversity.

1.2 Statement of the Problem and Study Justification

The genetic diversity of plant species is one of the important constituents of biological diversity. Plays an important role in protecting rare and endangered species mainly concentrated on the endemic species like *Urtica simensis* with restricted geographic distribution due to the potential of a species to respond adaptation in resist environmental changes and to the extent of genetic variability it contains.

Various studies of *Urtica simensis* have mainly focused on its medicinal uses, Polyphenol content with antioxidant properties, high nutrition value as well as useful chemical composition. Despite its plenty of use, genetic diversity information about *Urtica simensis* is limited. Hence, there is no enough information and literature available on the genetic diversity of *Urtica simensis* using molecular DNA markers and morphological characteristics. To conserve and choose glorious germplasm for the cultivation of *Urtica simensis* by the local communities and to maintain sustainable conservation policy, the study on the genetic diversity for this species is turning into necessary and timely with the help of Morphological characters and Molecular (ISSR) markers.

Consideration of morphological characters of plants encourages distinguishing, choosing preferred characteristics. This helps in exchanging their preferable genes in plants that are resistant to abiotic and biotic stresses through biotechnological and molecular techniques

for the increment of the significance of the germplasm (Tar'an *et al*., 2005). In addition, DNA markers (ISSR) markers are also used in genetic diversity studies in different crop plants which can detect polymorphisms without any previous knowledge of the crop's DNA sequence.

Therefore, this is important to evaluate the diversity of *Urtica simensis* to address and fill the gap in its morphological characterization, genetic variability, and distribution. Accordingly, this study evaluated the overall Morphological characterization and genetic variability of *Urtica simensis* which will be of great importance for the detection of valuable genetic resources and provide an important basis for their conservation and very useful to plan sustainable conservation policy.

1.3 Study Objectives

1.3.1 Overall objective

Evaluate and characterize the diversity of stinging nettle (*Urtica simensis*) in northern Ethiopia using morphological characterization and molecular marker techniques.

1.3.2 Specific objectives

1. To characterize *Urtica simensis* species of different sites/areas based on morphological characteristics.
2. To evaluate the genetic variation within and among *Uritica simensis* species.
3. To provide knowledge regarding conservation issues/strategies in cultivation and best use of genetic resources of *Uritica simensis* species.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Taxonomic Classification of Samma (*Uritica simensis*)

Urtica simensis belongs to Kingdom- *Plantae*; Phylum- *Magnoliophyta*; Class- *Magnoliopsida*; Order- *Urticales*; Family- *Urticaceae* and Genus- *Urtica*. The family *Urticaceae* is commonly known as nettle family comprises with the list of 48 genera and more than 2000 species of plants. Geographically, the species of these plants are mostly found in the tropical and subtropical regions of the world. While, 30-45 species of these plants are found within the cosmopolitan distribution of temperate regions. The genus *Urtica* comes from Latin word “Urere” which means “burn”. Nettles are often easily recognizable for humans after having experienced for its sting (Gebrezgabiher *et al.*, 2013; Sharma *et al.*, 2015; Wu *et al.*, 2015).

2.2 Morphological Characterization of Plants

The analysis of genetic variation both within and among plant materials is the main concern to plant. Varieties of methods including morphological characterization in presence of morphological traits such as plant height, leaf shape, flower and fruit colour, stem and length. These are among the traits/markers that have been frequently used in the evaluation of genetic variability in some plant species because of they come up with an easy way of quantifying genetic variation while examine genotype performance under growing environments (Fufa *et al*., 2005) and which is not implemented in characterizing diversity of *Urtica simensis*.

Morphological characters are useful in separating between closely related germplasm sources and could have applications in genetic diversity studies of endemic species like

Urtica simensis, identifying unknown origins of germplasm sources, plant variety protection, and/or cultivar identification and in addition can be used in breeding programs aimed at improving the crop for various traits of economic importance (Cortese *et al*., 2010; Karuri *et al*., 2010). They have been utilized in plenty of plant genetic diversity studies of various plant species to list some of them in Tomato (Naz *et al*., 2013), *Cinnamomum burmanii (*Lizawati *et al*., 2018), *Mentha* Species (Shinwari *et al*., 2011), *Lens culinaris* Medikus ssp. *Culinaris* (Ahamed *et al*., 2014), *Trifolium hybridum* (Paplauskienė and Dabkevičienė, 2012), *Achillea santolina* (Abdelfattah *et al.*, 2014), *Panicum virgatum* L. (Cortese *et al*., 2010) and sweet potato (Karuri *et al*., 2010).

2.3 Ethno Botanical Uses of *Uritica simensis*

Urtica simensis has medicinal use, in Ethiopia people traditionally used different parts of the plant to cure different ailments and disorders. Root, fresh leaves and young twigs are used for the treatment of gonorrhea, gastritis as well as play an important role in control of bacterial and fungal infections and acute stomachache, body swelling and common cold, Rh-factor and heart failure (Gebrezgabiher *et al.*, 2013; Enyew *et al.*, 2014; Alemayehu *et al.*, 2015; Kefalew *et al.*, 2015). In addition it is used for therapeutic function and the most common use of the leaf extracts of *Urtica simensis* in Ethiopian traditional medicine used for the treatment of diabetes (Tsegaye *et al.*, 2009).

2.4 Nutrient and Chemical Composition of *Uritica simensis*

Urtica simensis (Samma) is rich indigenous nutrient source which increases the intake of pro- vitamin A, calcium, potassium, iron and zinc and also Stinging nettle or *Urtica simensis* Leaf Meal can partly replace concentrate mixture in dairy goat ration without undesirable effect on lactation performance (Keflie *et al.*, 2017). Its crude protein content

(27%) computable with of high protein legumes like Alfa Alfa (Andualem *et al.,* 2015). It has high nutritional value compared to many green leafy vegetables commonly cultivated and consumed in Ethiopia and its mineral content is exceptionally high which makes this vegetable as an inexpensive but high quality nutrition source (Assefa *et al.,* 2013) and leaves is a potential source of both for macro and micro-nutrients for human food (Alemayehu *et al.,* 2016). *Urtica simensis* leaves contain appreciable amount of phytochemical constituents (phenolics, flavonoids and tannins) considered as an easily accessible and valuable natural source of antioxidants and dietary supplement (Seifu *et al.,* 2017).

2.5 Molecular Markers and Plant Genetic Diversity

Molecular markers are most widely used DNA analysis based markers inherently take place polymorphism detected DNA sequence, obtained at specific positions of the genome and connected with the character of a trait or linked gene (Thottappilly *et al*., 2000; FAO, 2004). They take a crucial part in preservation of biodiversity, establishment of encouraging cultivars, quantitative trait loci (QTL) mapping (Khanam *et al*., 2012). They are highly robust implemented tools in the investigation of genetic variation and in elucidation of genetic relationships within and among species (Chakravarthi and Naravaneni, 2006).

Plant genetic diversity is an important particular area in constituting and protecting of biodiversity. Plays crucial role in solving of food insecurity in developing countries. Diversity of plants based on phenotypic and morphological characters, which fluctuate with environmental conditions and evaluation of characters requires time until the plant grow up and become matured. However, to overcome such problems and fluctuations the expansion of Biotechnology and molecular biology admit effortless assessment of huge

number of loci allocate throughout the genome of the plants such as molecular markers (Jonah *et al*., 2011).

The genetic diversity of plant species can be assessed through various types of molecular markers which have been reported as highly polymorphic and reproducible, such as restriction fragment length polymorphisms (RFLPs), amplified fragment length polymorphisms (AFLPs), simple sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs).

RFLPs are highly reproducible markers which need a large amount of DNA, laborious, and mostly require radioactively labeled probes or primers (Agarwal *et al.*, 2008). AFLPs are highly reproducible markers; this enables rapid generation and high frequency of identification of polymorphic loci. In addition, this makes an attractive technique for identifying polymorphisms and for determining linkages by analyzing individuals from a segregating population. They are expensive and also require high-resolution electrophoresis or automated sequences (Mohan *et al*., 1997).

SSR also known as Microsatellites are expensive and require high-resolution electrophoresis or automated sequences markers which are short tandem repeats, their length being 1 to 10 bp, are highly variable and evenly distributed throughout the genome, their number of repeated units fluctuating widely between crop species (Queller *et al.*, 1993).

SNPs are DNA sequence variations that take place when a single nucleotide (A, T, C, or G) in the genome sequence is altered, which leads dissimilarity in genome sequence of individuals of a population and these polymorphisms are single-base substitutions among

sequences. Those markers are less laborious than the rest of the markers and highly controlled to automation, the cost that require is very high (Jehan and Lakhanpaul, 2006; Govindaraj *et al*., 2015).

2.6 Inter-simple Sequence Repeats (ISSRs) Markers

Inter-simple sequence repeats (ISSRs) and random amplified polymorphic DNA (RAPD) considered as an alternatives because of the lower level of skill required, low cost per assay, and the ready availability of primers allow the scanning of the entire genome and efficient genotype characterization. Therefore, because of their characteristics and efficiency for detecting polymorphisms, the ISSR and RAPD markers have been successfully used to evaluate the intra or inter-specific genetic diversity in different domestic and wild species (Muthusamy *et al.*, 2008).

ISSR (inter-simple sequence repeat) are multi locus markers has a few advantages over other markers because ISSR primers are quick, easy to apply, highly reproducible and polymorphous, anneal directly to simple sequence repeats and no need of prior genetic sequence information (Godwin *et al.,* 1997; Bornet and Branchard, 2001). They have longer primers, allow more stringent annealing temperatures and reveal more polymorphic fragments, can be highly variable within a species than RAPD (random amplified polymorphic DNA) markers and have more polymorphic information content (PIC) as well as required less cost of analyses than AFLP (amplified fragment length polymorphism) and RFLP (restricted fragment length polymorphism) (Tsumura *et al.,* 1996; Fang and Roose, 1997; Nagaoka and Ogihara, 1997; Esselman *et al.,* 1999; Goulão and Oliveira, 2001).

Hence, ISSR has been widely utilized in population genetic studies of various plant species, including many perennial and medicinal plants such as in *Urtica dioca*

(Haghpanah *et al.*, 2016), *Lepidiumsativum* (Hassen, 2018), *Aframomum corrorima* (Chombe and Bekele, 2018), *Malus*sp. (Goulão and Oliveira, 2001), *Lobelia* sp. (Geleta *et al.*, 2009), *Dioscoreaalata* (Wu *et al.*, 2009), mulberry (Kalpana *et al.*, 2012), *Populuscathayana* (Lu *et al.*, 2006), *Psammochloavillosa*, *Prunus subgenus* (Shahi *et al.*, 2011), O *ryzasativa* (Blair *et al.*, 1999), *Punicagranatum* (Narzary *et al*., 2010), *Phaseolus vulgaris* (Galvan *et al.*, 2003) and *Changium smyrnioides* and *Chuanminshen violaceum* (Qiu *et al.*, 2003).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of Study Areas and Sample Size

This study was carried out in two areas i.e. Mekelle (Tigray) and Gondar (Amhara) Northern Ethiopia, with specific districts of sample collected known as (Quiha and Fasiledes respectively) (Fig. 2) which are known for their high potential in *Urtica simensis* production.

Mekelle is the capital city of Tigray Region located 780 km north of Addis Ababa, the capital city of Ethiopia. Its geographic location is 13˚49" N and 39˚ 47" E. It has an average altitude of 2254 meters above sea level with annual rainfall is 530 mm and an average temperature of 18ºC or 64.4˚F. The total population is close to 310 436. Gondar is located at 12˚60" N and 37˚46" E which is around 727 km from Addis Ababa with an altitude of 2 201 meters above sea level and annual rainfall is 1151 mm/year and an average temperature of 22.0ºC or 71.6˚F. The total population is close to 310 000. The Molecular characterization experimental study was carried out at the Molecular Biology laboratory at Sokoine University of Agriculture, Tanzania.

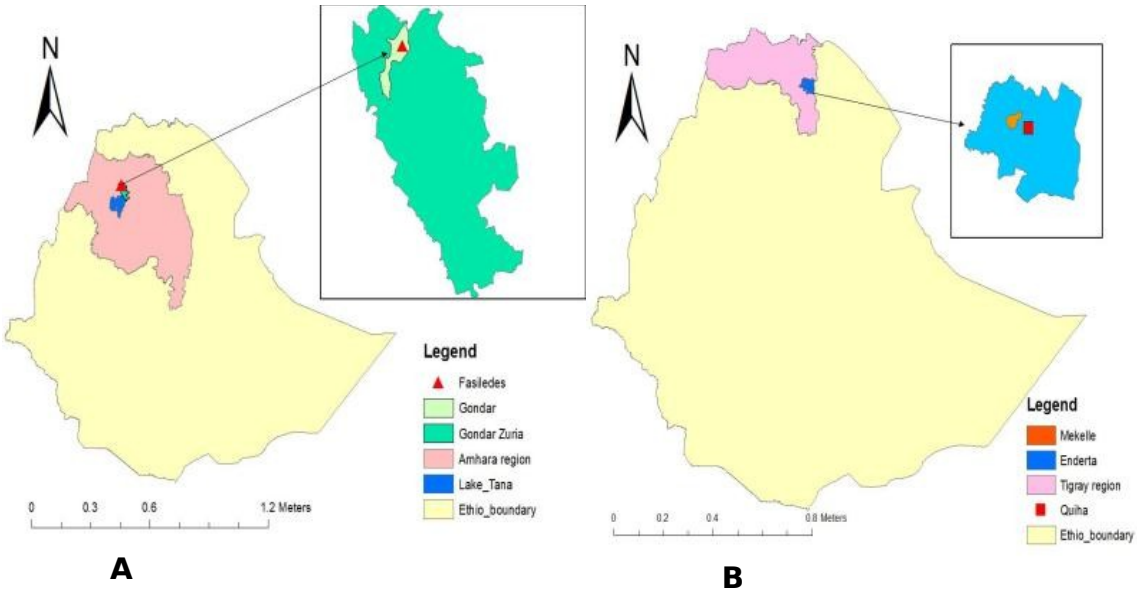


Figure 2: Map of Ethiopia; A. Gondar and B. Mekelle, showing study areas of Northern Ethiopia

3.2 Field Survey and Sample Collection

Field surveys were conducted in the aforementioned Northern, areas of Tigray (Mekelle) and Amhara (Gondar) region, Ethiopia. Followed by purposive sampling methods and selection of study areas, based on the availability of different targeted morphological appearances which are helpful in the evaluation of morphological diversity analysis and genetic variability and also based on production potentials of the study areas according to (Lizawati *et al*., 2018). A total of 133 (65 from Gondar and 68 from Mekelle) young leave samples were selected and collected using poly bags on basis of targeted morphological appearances as shown in (Appendix 1).

3.3 Plant Material

Urtica simensis plant species were collected from considerable growing areas i.e. Gondar and Mekelle northern part of Ethiopia. Based on society/community prior knowledge on its importance and availability in addition to its population. A total of six different quantitative

(two) and qualitative (four) morphological characters were evaluated and recorded with two replications from fresh plant material in the field and ten plants were randomly selected for scoring according to studies conducted/reported by (Figueredo-Urbina *et al*., 2017; Lizawati *et al*., 2018).

3.4 Morphological Characterization

Morphological characters of *Urtica simensis* like Plant Height (PH), Stem Length (SL), Flower Color (FC), Leave Shape (LS), Leave Arrangement (LA), and Plant Growth Habit (PGH) were performed during field survey (Appendix 1). Those morphological traits were classified into two quantitative and four qualitative traits. The two quantitative characters such as plant height and stem length were measured in cm from ground level to the top of spike for plant height and from the soil level to end of flower part for stem length with meter stick respectively. The four qualitative characters like flower color, leaf arrangement, leaf shape, and plant growth habit were recorded and scored following the morphological descriptors (Appendix 2) reported on the International Union for the Protection of New Varieties of Plants (UPOV, 2006) for the scoring of flower color, following the study reported by (Chaki *et al*., 2018 (in press) as a base for the scoring of leaf shape and leaf arrangement and (Bioversity International, 2007) for the scoring of plant growth habit.

3.5 DNA Extraction and Qualification

Young leaves of each *Urtica simensis* sample (two to three grams) were harvested and lyophilized then ground using mortar and pestle and stored in Eppendorf tube/PCR tube (Inqaba, Nairobi, Kenya), sample preparation, DNA extraction was performed DNA isolation kit (Terzopoulos and Bebeli, 2008) and instructions using (Inqaba, Nairobi, Kenya). The quality of the extracted DNA was measured by running on a 0.8 % agarose

gel for the good separation of large DNA fragments and the gel was stained using Ethidium bromide solution. Appropriate dilutions of DNA were made for further amplification and ISSR analysis.

3.6 ISSR Amplification and Gel Electrophoresis

Among sixteen random primers (Haghpanah *et al.*, 2016) utilized, only five well-amplified primers showing polymorphic results were selected for diversity and similarity analysis. ISSR assays were performed using PCR kit or beads (Bioneer, Korea) which is PCR premix in a final volume of 20 µl containing 2 µl template DNA, 2 µl random primer, and 16 µl PCR water. The mixture was amplified in a thermal cycler that was programmed for one cycle of initial denaturation at 94^{o}C for 5 min, 35 cycles of 94^{o}C for one min, followed by specific annealing at 50^{o}C (based on the primers annealing temperatures) for 1 min, and ending with an extension at 72^{o}C for 1 min; and a final extension cycle at 72^{o}C for 5 min. The PCR machine was adjusted to hold the product at 4^{o}C. The PCR products and 1kb DNA ladder were electrophoresed on 1.8% agarose gel for the resolution of small DNA fragments (stained with Ethidium bromide) and photographed under Gel documentation system.

3.7 Data Analysis

Principal component analysis (PCA) was carried out using the original numerical data of quantitative characters and the assigned qualitative trait data and excluding the constant variables with similar morphological appearances among the populations using R studio software. Jaccard’s coefficient similarity was measured and a dendrogram based on similarity coefficients was generated by the Unweighted Pair Group Method of Arithmetic means (UPGMA) cluster analysis using NTSYS software (Rohlf, 2000).

The banding patterns obtained from ISSR were scored as present (1) or absent (0). POPGENE software was used to calculate Nei's (1978) unbiased genetic distance- among different cultivars with all markers, including monomorphic markers and genetic parameters including genetic diversity for each population as the number of polymorphic loci, percent polymorphism, Genetic diversity (H), and Shannon diversity index (I). An analysis of molecular variance (AMOVA) pair-wise procedure was used to calculate the genetic variance among and within-population or varieties.

CHAPTER FOUR

4.0 RESULTS

4.1 Analysis on Morphological Characterization and Genetic Diversity of *Urtica simensis*

4.1.1 Quantitative Appearances Analysis

Results of the analysis of morphological traits showed significant ($p < 0.001$) differences were found among samples for all the measured traits, indicating levels of variation in the studied quantitative morphological traits. Mean, range, standard deviation, and percentage of coefficient of variation (CV) were calculated for the two quantitative morphological traits PH (Plant Height) and SL (Stem Length) of populations (Mekelle and Gondar) as presented in (Table 2). PH (range from 80 cm to 200 cm) was recorded from Gondar while PH value (range from 25 cm to 160 cm) was recorded in Mekelle and Regarding SL value (range 50 cm to 80 cm) was recorded in Gondar and the SL values recorded in Mekelle (range from 25 cm to 50 cm) (Table 2).

Table 2: Descriptive Analysis of two quantitative morphological Appearances of *Urtica simensis*

Components	Sample location	Plant height (PH in cm)	Stem length (SL in cm)	Over all (133 No. of samples)	
				Plant height	Stem length
Min	MK	25.0	20.0	25.0	20.0
	GD	80.0	50.0		
Max	MK	160.0	50.0	200.0	80.0
	GD	200.0	80.0		
Mean	MK	102.8	37.0	124.3	50.2
	GD	146.8	64.0		
Std Dev (SD)	MK	47.0	8.8	50.3	16.4
	GD	43.6	9.8		
Variance (S^2)	MK	2207.7	77.1	2527.3	269.4
	GD	1897.2	97.0		
CV, %	MK	45.7	23.8	40.5	32.7
	GD	29.7	15.3		

Min. = Minimum value, Max. = Maximum value, GD = Gondar, MK = Mekelle

% CV = SD/mean * 100

Where, CV = Coefficient of variance, SD = Std Deviation

4.1.2 Qualitative morphological appearances analysis

Qualitative morphological traits of *Urtica simensis* among the populations of Mekelle and Gondar were recorded and scored (Appendix 1 and 2) according to standard descriptors. The morphological appearances of Leaf Shape (LS), Leaf Arrangement (LA), and Plant Growth Habit (PGH) in 133 of total sample size were lenceolate, opposite and erect respectively with 100 % similarity between the populations (Appendix 1 and 2), whereas the trait Flower Color (FC) showed variation in both study areas (Table 3). Due to the variation and ability to create a diversity of this trait (FC) compared to the other recorded traits were included during correlation coefficient analysis for the formation of the

correlation matrix and PCA analysis for the calculating variations among the targeted morphological traits.

Table 3: Analysis of qualitative morphological trait (Flower Color) of *Urtica simensis* in percentage

Sample location	Amount of samples	Flower Color (FC) representative	Percent %	Cumulative percent	Total
MK	30	6	44.12	44.12	68
	38	46	55.88	100	
GD	34	9	52.31	52.31	65
	31	48	47.69	100	

MK= Mekelle GD= Gondar

4.1.3 Correlation coefficient matrix and principal component analysis

The Pearson correlation coefficient output data indicated statistically significant correlations between the three morphological recorded variables (Table 4). PH and SL were positively correlated, and PH and FC were negatively correlated, as well as SL with FC was also negatively correlated and this correlation matrix result was the base for performing in the PCA analysis.

Table 4: Correlation Matrix among the three morphological characters

	PH	SL	FC
PH	1.000		
SL	.533	1.000	
FC	- .426	- .754	1.000

Significant at p – value =0.00, PH = Plant Height, SL = Stem Length and FC = Flower Color

The PH, SL, and FC showed negative vector values and this indicated that all the traits negatively correlated with PC1. In PC2, only SL loaded; In PC3, SL was highly loaded following FC with eigenvector of 0.701 and 0.102 respectively (Table 5). This indicates the PC3 reflected as a considerable component of plant species indicator. Based on the total

variations among these major morphological traits expressed in Scree plot (Fig. 3) which represented the data output of eigenvalue versus the number of Components (Principal components) and indicated the retained amount of principal components.

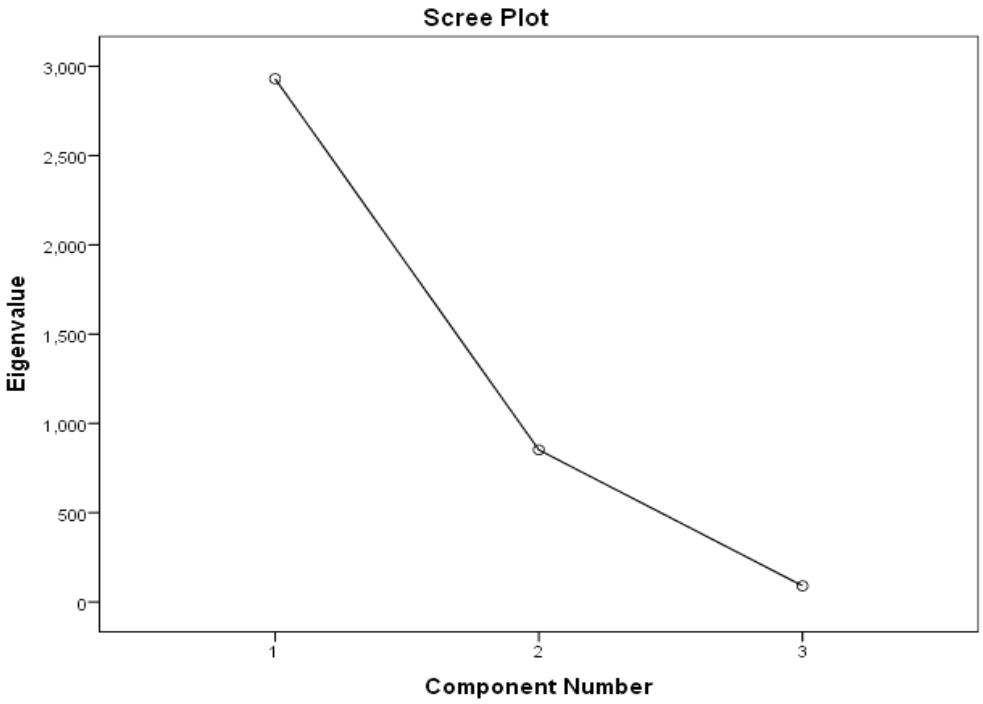


Figure 3: Data Representation using Scree plot based on eigenvalue among the major morphological traits

Table 5: Principal Component Analysis based on Covariance Matrix and total variance

	PC1	PC2	PC3
Plant Height	-0.708	-0.019	-0.706
Stem Length	-0.702	0.125	0.701
Flower Color	-0.075	-0.992	0.102
Eigen values	2 929.80	851.66	90.38
% of Variance	75.669	21.996	2.334
Cumulative, %	75.669	97.666	100.000
Std dev.	1.239	1.001	0.679
Prop. of Variance	0.512	0.334	0.154
Cumulative Prop.	0.512	0.846	1.000

4.1.4 Cluster Analysis of *Urtica simensis* based on the morphological analysis data

Based on morphological character recorded data results dendrogram cluster analysis (Fig. 4 and Appendix 3) and the mean value of each group (Table 6), concerning the values of coefficient of variances (CV) evaluated *Urtica simensis* traits were grouped into six major clusters with significant differences in the morphological characteristics and intermixing of individuals of both populations in morphological characters and location. The sub-clusters at the same line within the major and minor clusters are associated with the same morphological appearance like plant growth habit (PGH), leaf shape (LS), and leaf arrangements (LA).

Cluster I contained 20 sample traits collected only from Mekelle. The main features were PH between 25.0 and 160.0 cm, and SL between 20.0 and 50.0 cm with a mean value of PH and SL 95.5 and 37.4 respectively with a respect CV value of PH 52.0 % and SL 24.3 %.

Cluster II contained 17 sample traits collected from Mekelle (1) and Gondar (16), with a PH between 50.0 and 200.0 cm, and SL between 45.20 and 80.0 cm. with a mean value of 141.2 and 63.1 respectively and PH 32.6 % and SL 19.5 % of CV value.

Cluster III consisted of 24 sample traits collected only from Gondar. The main features were PH between 80.0 and 200 cm, and SL between 50.0 and 80.0 cm with a mean value of PH and SL 147.9 and 63.6 respectively 29.6 % PH and SL 13.1 % value of CV.

Cluster IV consisted of 21 individual traits only from Mekelle. The main features were PH between 25.0 and 160.0 cm, and SL between 20.0 and 50.0 cm with a mean value of PH and SL 106.0 and 36.8 respectively, 39.2 % of the CV of PH and SL CV value of 24.2 %. Cluster V had 16 traits collected from Mekelle (5) and Gondar (11) province. With a PH between 80.0 and 200.0 cm, and SL between 33.90 and 80.0 cm. with a mean value of 143.1 and 55.4 respectively with CV values of 28.4 % PH and 24.2 % of SL.

Cluster VI had 35 traits, and this group was collected from Mekelle (22), and Gondar (13). With a PH between 25.0 and 200.0 cm, and SL between 21.9 and 80.0 cm. as well as mean values of 119.1 and 47.0 respectively with 46 % PH and 38.1 % SL of CV values. The traits with their respective individuals consisted of each cluster indicated in (Appendix 3).

Table 6: Overall cluster analysis among the morphological traits

Component	Sample traits	Clusters (Groups) Number I	II	III	IV	V	VI
Mean	PH	95 5	141.	147.9	106.0	143.1	119.1
	SL	37 4	2	63.6	36.8	55.4	47.0
Std Dev.	PH	49.7	63.1	43.8	41.6	40.6	54.8
(SD)	SL	9.1	45.9	8.3	8.9	13.4	17.9
	PH	52.0	12.3	29.6	39.2	28.4	46.0
CV, %	SL	24 3	32.6	13.1	24.2	24.2	38.1
			19.5				

PH = Plant Height, SL = Stem Length

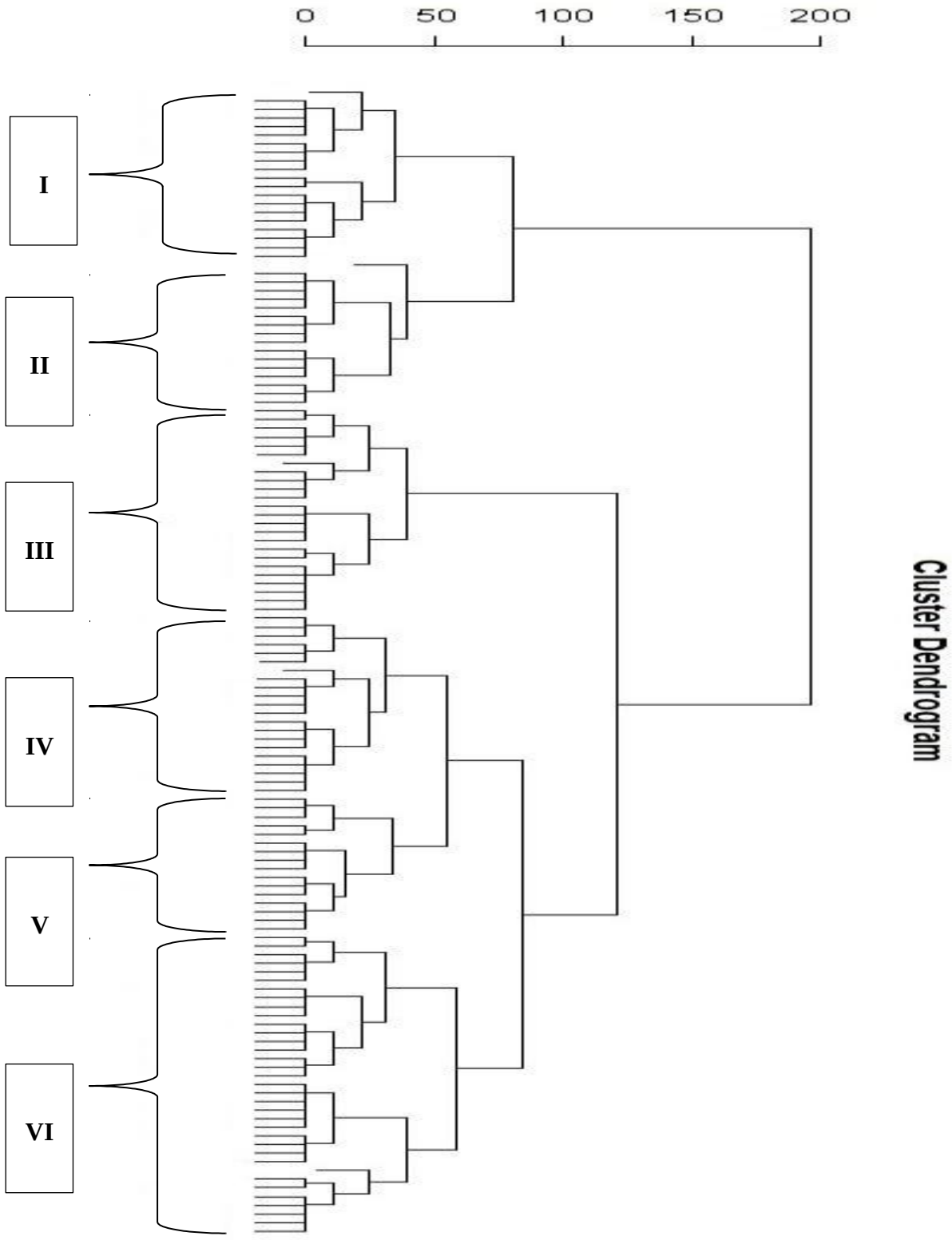
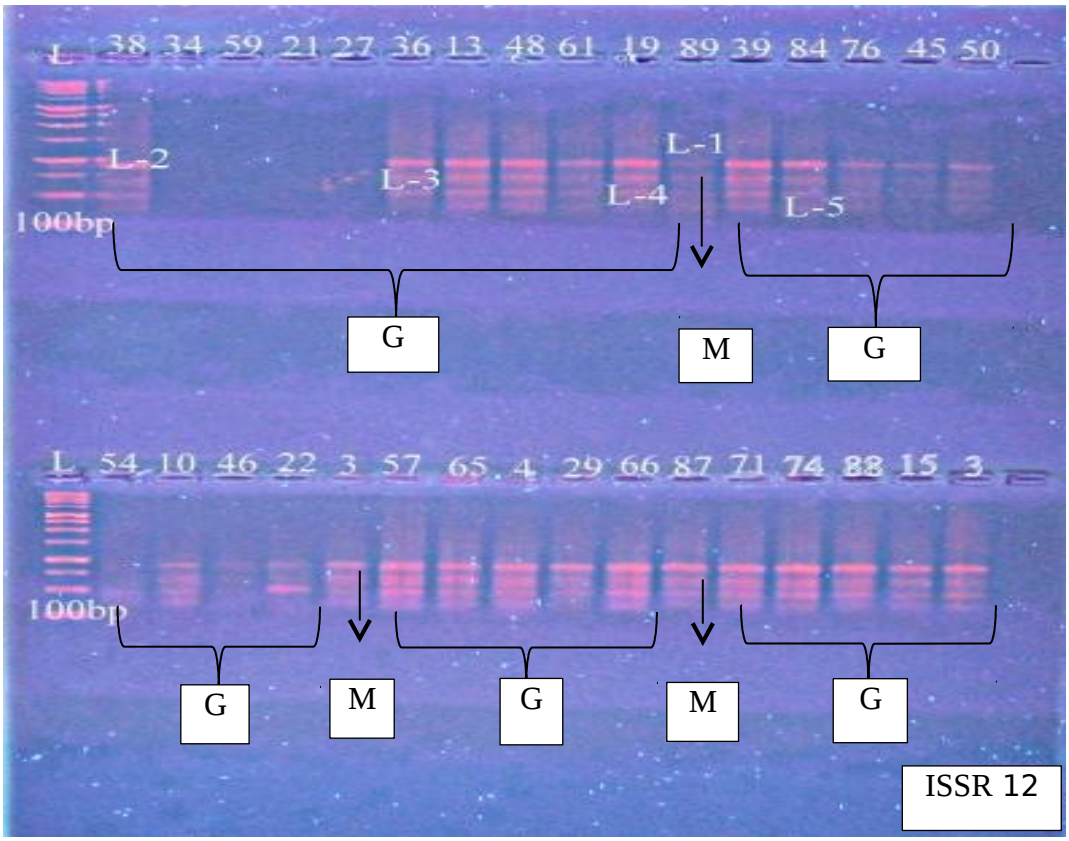


Figure 4: **UPGMA dendrogram Cluster analysis of *Urtica Simensis* generated using morphological characters showing six major clusters**

4.2 Analysis of Molecular Genetic Diversity of *Urtica simensis*

4.2.1 Genetic diversity analysis among the ISSR markers

Molecular diversity analysis of 133 *Urtica simensis* samples was determined using ISSR markers. The results expressed in terms of Number of Amplified Bands (NAB), Number of Polymorphic Locus (NPL), Nei (genetic diversity) values (H), percent of polymorphism (PP), and Shannon information index (I) based on five ISSR primers and diversity among the two study areas of northern Ethiopia (Table 7) and Table (8). The highest and good number of amplified bands was recorded primers ISSR12, ISSR4, ISSR14, and ISSR 8 (111, 106, 99, and 75 respectively), while the lowest number of amplified bands was recorded primer ISSR13 (54). Primer ISSR4 and ISSR13 recorded the same Number of Polymorphic Locus (6), the higher number of Polymorphic Locus was registered at primer ISSR8 (8) and ISSR12 and ISSR14 were scored 5 Polymorphic Locus (Table 7).



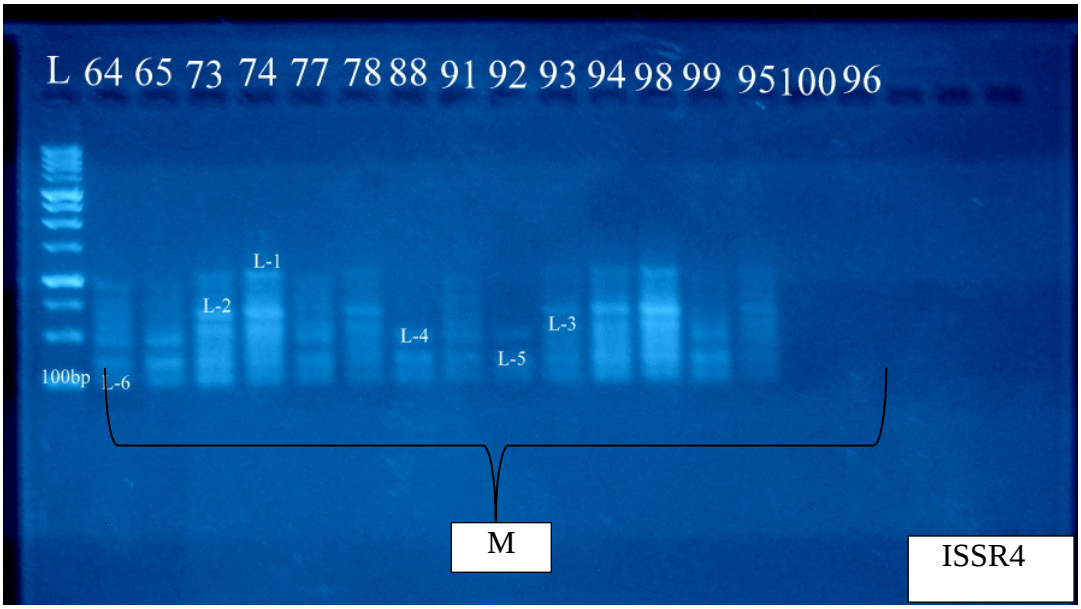


Figure 5: Representatives for gel electrophoresis PCR product of ISSR markers which shows Polymorphism with Number of Polymorphic Locus (G= samples from Gondar, M= samples from Mekelle)

Table 7: General Diversity analysis of *Urtica Simensis* based on Molecular (5 ISSR) markers

Primers	Sequence(5'→3')	NAB	NPL	PP (%)	H	I
ISSR4	$GA)_8C$	106	6	100	0.38	0.55
ISSR8	$(GA)_8A$	75	8	100	0.29	0.45
ISSR12	$(TG)_8A$	111	5	100	0.36	0. 54
ISSR13	$(AC)_8C$	54	6	100	0.42	0.59
ISSR14	$(ATC)_6T$	99	5	100	0.43	0.62
Total		**445**	**30**	**100**	**1.88**	**2.75**

NAB= Number of Amplified Bands, NPL= Number of Polymorphic Locus, H =Nei (genetic diversity) values, PP (%) = percent of polymorphism and I=Shannon information index.

The present study revealed that out of 16 ISSR markers only 5 primers (markers) were showing polymorphism. 30 total numbers of polymorphic loci were produced by 5 primers. Primer ISSR14 showed the highest genetic diversity and Shannon Information Index (0.43 and 0.62 respectively) following by primer ISSR13 which showed (0.42 and 0.59 respectively) and least genetic diversity and Shannon Index was produced by ISSR8 (0.29 and 0.45 respectively) (Table 7).

4.2.2 Molecular based genetic diversity among populations

All the five primers were polymorphic in both populations/study areas (Tables 7 and 8). The highest genetic diversity was indicated in samples from Mekelle compared to samples from Gondar (0.3462 (34.62%) and 0.3152 (31.52 %) respectively) with respect to 3.10 % of difference diversity value (H) as shown in (Table 8).

Table 8: Genetic diversity of *Urtica simensis* based on ISSR results among the two study areas (populations)

Populations code	NPL	PP (%)	H	I
GD	25	100	0.3152	0.484
MK	29	100	0.3462	0.502

Number of Polymorphic Loci (NPL), Percent Polymorphism (PP), Genetic diversity (H) and Shannon information index (I)

4.2.3 Cluster analysis of *Urtica simensis* based on the molecular (ISSR) markers

The Dendrogram based on Jaccard's similarity coefficients generated by UPGMA (Fig. 6). Cluster analysis of ISSR recorded data of all 133 samples without grouping the data with respect to locations generated three major clusters (Fig. 6). The broad distribution of *Urtica simensis* individuals over the entire tree which indicated low divergence among populations of both study areas.

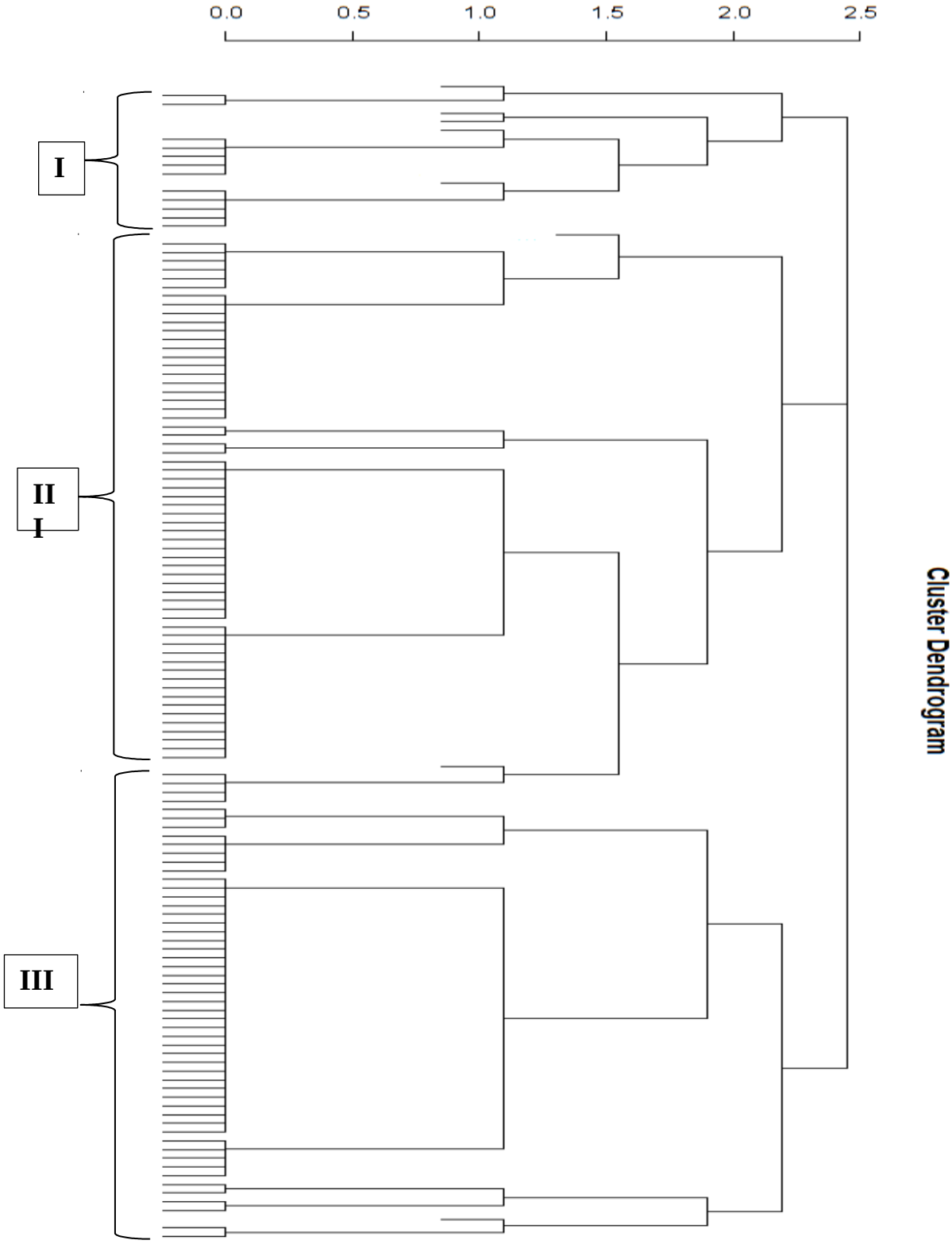


Figure 6: UPGMA dendrogram Cluster analysis of *Urtica Simensis* generated using Five ISSR markers showing three major clusters

CHAPTER FIVE

5.0 DISCUSSION

Approachable genetic diversity in genetic resource collections can highly make possible well-founded classification and determination of main traits with possible importance in maintaining biodiversity (Majidi *et al*., 2009). The results of this study showed that both populations (Mekelle and Gondar) have variations in quantitative traits such as PH (Plant Height) and SL (Stem Length). The qualitative traits *Urtica simensis* morphological appearances like Leaf Shape (LS), Leaf Arrangement (LA), and Plant Growth Habit (PGH) of this study were similar in both study populations while the trait Flower Color (FC) showed some variations in both areas and this reflected the appearance of *Urtica simensis* species and used to detect the species from other species of the same genius with a combination of the quantitative traits PH and SL with consideration of the similarity of qualitative traits in both areas. Whereas the study reported by (Shen *et al*., 2018) on morphological diversity of Job's-tears (*Coix lacryma-Jobi* L.) revealed that morphological variation like PH(Plant Height), SNN(stem node number), and PBN (primer branch nodes) may reflect as the main factor of plant variety with respect to PCA.

As the results of this study showed that Plant Height, Stem Length, and Flower Color varied within individuals in the same area and also between the study areas and this indicated that each individual genetically unique in nature. Therefore, the variation of those both quantitative (controlled by diverse genes) and qualitative (controlled a couple of genes) targeted traits utilized in this study could be led to excellent or good gene resources conservation strategies due to its capacity in building diversity and its potential in providing implementations for population detecting and evaluation that could be used for conservation planning. Most of the morphological appearances recorded in this study

supported to the *Urtica simensis* morphological characters mentioned in Flora of Ethiopia book (Volume 3) (Friis, 1989) which has been reported as an erect, perennial herb its height reached up to 1 m tall in some places with opposite leave arrangement.

The analysis of simple correlations among the quantitative traits (PH and SL) and qualitative (FC) are significant and led to the generating of PCA to insist on variation and made a strong figure in a dataset. The results revealed that PH (Plant Height) and SL (Stem Length) traits, showed positive correlation could be used as competent selection basis or standard to evaluate traits whereas negative correlations were presented among the traits PH (Plant Height) with FC (Flower Color) and SL (Stem Length) with FC and this significant correlation among the qualitative and quantitative traits may need further studies.

Regarding PCA, it was generated based on the correlation coefficients matrix with eigenvalues which summarized variation among the measured traits of morphological appearances through components (Principal Component). The present study it is revealed that the three morphological traits (quantitative traits PH, SL and qualitative traits FC) of the 133 *Urtica simensis* formed three Components, and the morphological traits PH, SL, and FC were indicated negatively correlated in PC1 with negative eigenvectors and PC 2 loaded with SL trait with positive eigenvector which indicated a positive correlation. The PC3 loaded both with SL and FC morphological traits might be reflected as the main factor of plant species.

This present study on *Urtica simensis* showed the morphological traits (quantitative (Plant Height and Stem Length) and qualitative traits (Flower Color)) particularly Plant Height

were negatively correlated in all the three components which could be contradicted with the study conducted by (Esfandani *et al*., 2017) on species delimitation in *Geranium* L. Plant genius using Morphological and Molecular reported that Morphological characters like, bract width (quantitative trait) and mericarp surface, mericarp hair, seed shape, sepal hair (qualitative traits), the most variable morphological characters and positively correlated with three components with respect to 71.5% of the total variation.

Genetic diversity using morphological traits in a species is constrained through a number of natural factors including gene flow, and geographic range among the traits. The variations in morphological traits can be used to classify materials in different groups. According to the cluster analysis of this study morphological traits of 133 sampled traits showed closer resemblance among populations of *Urtica simensis* consisted of six clusters compared to ISSR polymorphism contained three clusters as indicated by the distributed mean values with their percentage of variance and distance on the trees distance scales.

Based on the study conducted on Genetic diversity of *Urtica dioca* using ISSR molecular markers (Haghpanah *et al.,* 2016) revealed that the highest Number of Amplified Bands and Number of Polymorphic Loci was produced in primer ISSR8 with respect to 72% polymorphism and compared to results of this current study and results by (Haghpanah *et al.*, 2016) revealed that, the percentage polymorphism ranged from 17% to 92% among 16 primers and 68% average polymorphism was reported in genetic diversity of nettle plant *Urtica dioca*. Whereas this study revealed that ISSR12 produced the highest Number of Amplified Bands and ISSR8 shown the highest NPL with respect to total 100% average polymorphism, but all the primers with samples collected from both Northern parts revealed that high percent of polymorphism(100%) and genetic diversity as well compared to *Urtica dioca*.

UPGMA analysis based on both locations (Gondar (Amhara) and Mekelle (Tigray) regions) with individuals appears intermixing of individuals to different groups. Analysis of Molecular Variance (AMOVA) of this study conducted on overall ISSR data recorded on *Urtica simensis* variations was demonstrated within individuals in each population rather than among populations (in both study areas).

This study is consistent with the study carried out on genetic diversity of *Mentha* species (Shinwari *et al.*, 2011) which showed that high levels of genetic polymorphism or variation were recorded among species or individuals rather than among populations and the polymorphism within populations which represented genotype richness, recombination, and gene flow.

A study reported by Hassen (2018) in genetic diversity of *L. sativum* using ISSR markers and morphological characters revealed that the dendrogram formed four clusters and two sub-clusters of *L. sativum* with samples collected from five different regions including Amhara and Tigray regions (formed in the first cluster) and UPGMA shows intermixing of individuals to different groups.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

This study revealed some variations in *Urtica simensis* collected from the study areas, demonstrated diversity among populations (in both study areas) and within individuals in the same study area and this may due to the morphological appearances related to geographical or environmental differences and genetic variability (biased capacity of utilized markers or primers). ISSR markers like (ISSR12 and ISSR8) utilized in this study in evaluating genetic diversity of *Urtica simensis* the results indicated that they are effective markers in evaluating genetic diversity of *Urtica simensis* with the potential of producing polymorphic bands, therefore they are useful for further marker-based studies of *Urtica simensis* and this has a valuable effect on the characterization of *Urtica simensis* genetic resources in different parts of Ethiopia, could be used in providing knowledge and generating information useful for improvement and conservation policies of *Urtica simensis* which would expand in the natural population size as well as optimization and refinement of cultivation habits secured constant providing of *Urtica simensis* without utilizing the natural populations.

According to the findings of this study, the PCA indicates genetic diversity or variation in *Urtica simensis* in the presence of some morphological appearances such as Plant Height, Stem Length, and Flower, and they significantly correlated to each other and this correlation among these quantitative and qualitative traits need further studies. Based on the results of this study; it can be also recommended that further studies on this species should be conducted using other morphological characters like stem diameter, the thickness of the leaf, leaf length, leaf width, number of branches, average branch length,

ratio Leaf width, and such traits and also using other molecular markers and should be conducted genetic profiling (DNA sequencing) to solve the limited knowledge available on the level of genetic diversity of *Urtica simensis* genetic resource in Ethiopia.

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APPENDICES

Appendix 1: Morphological appearances qualitative data set

Sample Codes	Location	LS	LA	FC	PGH
29G	Gondar	Lanceolate	Opposite	Grey	Erect
66G	Gondar	Lanceolate	Opposite	Brown green	Erect
87G	Gondar	Lanceolate	Opposite	Grey	Erect
74G	Gondar	Lanceolate	Opposite	Brown green	Erect
88G	Gondar	Lanceolate	Opposite	Grey	Erect
15G	Gondar	Lanceolate	Opposite	Grey	Erect
3G	Gondar	Lanceolate	Opposite	Brown green	Erect
54G	Gondar	Lanceolate	Opposite	Brown green	Erect
10G	Gondar	Lanceolate	Opposite	Grey	Erect
46G	Gondar	Lanceolate	Opposite	Brown green	Erect
22G	Gondar	Lanceolate	Opposite	Grey	Erect
57G	Gondar	Lanceolate	Opposite	Brown green	Erect
65G	Gondar	Lanceolate	Opposite	Grey	Erect
4G	Gondar	Lanceolate	Opposite	Brown green	Erect
61G	Gondar	Lanceolate	Opposite	Brown green	Erect
19G	Gondar	Lanceolate	Opposite	Grey	Erect
39G	Gondar	Lanceolate	Opposite	Grey	Erect
84G	Gondar	Lanceolate	Opposite	Brown green	Erect
76G	Gondar	Lanceolate	Opposite	Grey	Erect
45G	Gondar	Lanceolate	Opposite	Brown green	Erect
50G	Gondar	Lanceolate	Opposite	Brown green	Erect
38G	Gondar	Lanceolate	Opposite	Grey	Erect
34G	Gondar	Lanceolate	Opposite	Grey	Erect
59G	Gondar	Lanceolate	Opposite	Brown green	Erect
21G	Gondar	Lanceolate	Opposite	Grey	Erect
27G	Gondar	Lanceolate	Opposite	Brown green	Erect
36G	Gondar	Lanceolate	Opposite	Grey	Erect
13G	Gondar	Lanceolate	Opposite	Brown green	Erect
48G	Gondar	Lanceolate	Opposite	Grey	Erect
71G	Gondar	Lanceolate	Opposite	Brown green	Erect
64G	Gondar	Lanceolate	Opposite	Grey	Erect
47G	Gondar	Lanceolate	Opposite	Brown green	Erect
40G	Gondar	Lanceolate	Opposite	Grey	Erect
100G	Gondar	Lanceolate	Opposite	Brown green	Erect
98G	Gondar	Lanceolate	Opposite	Brown green	Erect
12G	Gondar	Lanceolate	Opposite	Grey	Erect
8G	Gondar	Lanceolate	Opposite	Grey	Erect
42G	Gondar	Lanceolate	Opposite	Brown green	Erect
35G	Gondar	Lanceolate	Opposite	Grey	Erect
18G	Gondar	Lanceolate	Opposite	Brown green	Erect
96G	Gondar	Lanceolate	Opposite	Grey	Erect
5G	Gondar	Lanceolate	Opposite	Brown green	Erect
20G	Gondar	Lanceolate	Opposite	Brown green	Erect
86G	Gondar	Lanceolate	Opposite	Grey	Erect
79G	Gondar	Lanceolate	Opposite	Brown green	Erect

4G	Gondar	Lanceolate	Opposite	Grey	Erect
56G	Gondar	Lanceolate	Opposite	Brown green	Erect
78G	Gondar	Lanceolate	Opposite	Brown green	Erect
85G	Gondar	Lanceolate	Opposite	Grey	Erect
80G	Gondar	Lanceolate	Opposite	Brown green	Erect
99G	Gondar	Lanceolate	Opposite	Grey	Erect
91G	Gondar	Lanceolate	Opposite	Brown green	Erect
25G	Gondar	Lanceolate	Opposite	Brown green	Erect
14G	Gondar	Lanceolate	Opposite	Grey	Erect
92G	Gondar	Lanceolate	Opposite	Grey	Erect
52G	Gondar	Lanceolate	Opposite	Grey	Erect
30G	Gondar	Lanceolate	Opposite	Grey	Erect
58G	Gondar	Lanceolate	Opposite	Brown green	Erect
77G	Gondar	Lanceolate	Opposite	Brown green	Erect
9G	Gondar	Lanceolate	Opposite	Grey	Erect
82G	Gondar	Lanceolate	Opposite	Grey	Erect
69G	Gondar	Lanceolate	Opposite	Brown green	Erect
1G	Gondar	Lanceolate	Opposite	Brown green	Erect
16G	Gondar	Lanceolate	Opposite	Brown green	Erect
89G	Gondar	Lanceolate	Opposite	Brown green	Erect
3M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
4M	Mekelle	Lanceolate	Opposite	Grey green	Erect
7M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
9M	Mekelle	Lanceolate	Opposite	Grey green	Erect
12M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
13M	Mekelle	Lanceolate	Opposite	Grey green	Erect
14M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
15M	Mekelle	Lanceolate	Opposite	Grey green	Erect
16M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
17M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
19M	Mekelle	Lanceolate	Opposite	Grey green	Erect
21M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
22M	Mekelle	Lanceolate	Opposite	Grey green	Erect
24M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
25M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
27M	Mekelle	Lanceolate	Opposite	Grey green	Erect
31M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
32M	Mekelle	Lanceolate	Opposite	Grey green	Erect
35M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
36M	Mekelle	Lanceolate	Opposite	Grey green	Erect
37M	Mekelle	Lanceolate	Opposite	Grey green	Erect
39M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
40M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
44M	Mekelle	Lanceolate	Opposite	Grey green	Erect
45M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
46M	Mekelle	Lanceolate	Opposite	Grey green	Erect
49M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
52M	Mekelle	Lanceolate	Opposite	Grey green	Erect
53M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
54M	Mekelle	Lanceolate	Opposite	Grey brown	Erect

47M	Mekelle	Lanceolate	Opposite	Grey green	Erect
58M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
59M	Mekelle	Lanceolate	Opposite	Grey green	Erect
64M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
65M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
73M	Mekelle	Lanceolate	Opposite	Grey green	Erect
74M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
77M	Mekelle	Lanceolate	Opposite	Grey green	Erect
78M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
88M	Mekelle	Lanceolate	Opposite	Grey green	Erect
91M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
92M	Mekelle	Lanceolate	Opposite	Grey green	Erect
93M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
94M	Mekelle	Lanceolate	Opposite	Grey green	Erect
95M	Mekelle	Lanceolate	Opposite	Grey green	Erect
96M	Mekelle	Lanceolate	Opposite	Grey green	Erect
98M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
99M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
100M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
84M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
90M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
34M	Mekelle	Lanceolate	Opposite	Grey green	Erect
69M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
82M	Mekelle	Lanceolate	Opposite	Grey green	Erect
56M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
57M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
70M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
76M	Mekelle	Lanceolate	Opposite	Grey green	Erect
86M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
87M	Mekelle	Lanceolate	Opposite	Grey green	Erect
81M	Mekelle	Lanceolate	Opposite	Grey green	Erect
72M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
62M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
68M	Mekelle	Lanceolate	Opposite	Grey green	Erect
75M	Mekelle	Lanceolate	Opposite	Grey brown	Erect
61M	Mekelle	Lanceolate	Opposite	Grey green	Erect
71M	Mekelle	Lanceolate	Opposite	Grey green	Erect
89M	Mekelle	Lanceolate	Opposite	Grey brown	Erect

PH = Plant Height, SL = Stem Length , FC = Flower Color, LS = Leaf Shape, LA = Leaf Arrangement and PGH = Plant Growth Habit

Appendix 2: General Morphological Appearances of 2 quantitative and 4 qualitative traits with their descriptors

Sample Codes	Location	PH	SL	LS	LA	FC	PGH
29G	Gondar	80	58.5	39	89	48	1
66G	Gondar	120	55	39	89	9	1
87G	Gondar	150	62.8	39	89	48	1
74G	Gondar	180	65	39	89	9	1
88G	Gondar	120	60	39	89	48	1
15G	Gondar	80	52	39	89	48	1
3G	Gondar	80	50	39	89	9	1
54G	Gondar	150	70	39	89	9	1
10G	Gondar	180	70	39	89	48	1
46G	Gondar	120	65	39	89	9	1
22G	Gondar	200	75.5	39	89	48	1
57G	Gondar	200	60	39	89	9	1
65G	Gondar	150	55	39	89	48	1
4G	Gondar	80	62	39	89	9	1
61G	Gondar	180	75.3	39	89	9	1
19G	Gondar	80	65	39	89	48	1
39G	Gondar	120	70	39	89	48	1
84G	Gondar	180	61	39	89	9	1
76G	Gondar	150	78	39	89	48	1
45G	Gondar	120	50	39	89	9	1
50G	Gondar	80	50	39	89	9	1
38G	Gondar	80	52	39	89	48	1
34G	Gondar	120	60	39	89	48	1
59G	Gondar	180	65	39	89	9	1
21G	Gondar	150	62.8	39	89	48	1
27G	Gondar	120	55	39	89	9	1
36G	Gondar	80	58.5	39	89	48	1
13G	Gondar	150	70	39	89	9	1
48G	Gondar	180	70	39	89	48	1
71G	Gondar	120	65	39	89	9	1
64G	Gondar	200	75	39	89	48	1
47G	Gondar	200	60	39	89	9	1
40G	Gondar	150	55	39	89	48	1
100G	Gondar	80	62	39	89	9	1
98G	Gondar	180	75	39	89	9	1
12G	Gondar	80	65	39	89	48	1
8G	Gondar	120	70	39	89	48	1
42G	Gondar	180	61	39	89	9	1
35G	Gondar	150	78	39	89	48	1
18G	Gondar	120	50	39	89	9	1
96G	Gondar	200	65	39	89	48	1
5G	Gondar	150	80	39	89	9	1
20G	Gondar	200	50	39	89	9	1
86G	Gondar	180	55	39	89	48	1
79G	Gondar	180	51.5	39	89	9	1
4G	Gondar	120	80	39	89	48	1
56G	Gondar	80	70	39	89	9	1
78G	Gondar	200	80	39	89	9	1
85G	Gondar	200	55	39	89	48	1
80G	Gondar	200	70	39	89	9	1

99G	Gondar	150	50	39	89	48	1
91G	Gondar	180	80	39	89	9	1
25G	Gondar	120	75	39	89	9	1
14G	Gondar	80	72	39	89	48	1
92G	Gondar	200	55	39	89	48	1
52G	Gondar	200	65	39	89	48	1
30G	Gondar	120	80	39	89	48	1
58G	Gondar	200	50	39	89	9	1
77G	Gondar	80	70	39	89	9	1
9G	Gondar	180	55	39	89	48	1
82G	Gondar	150	50	39	89	48	1
69G	Gondar	200	70	39	89	9	1
1G	Gondar	180	51.5	39	89	9	1
16G	Gondar	200	80	39	89	9	1
89G	Gondar	150	80	39	89	9	1
3M	Mekelle	25	20	39	89	46	1
4M	Mekelle	100	40	39	89	6	1
7M	Mekelle	50	32	39	89	46	1
9M	Mekelle	160	40	39	89	6	1
12M	Mekelle	160	50	39	89	46	1
13M	Mekelle	25	22	39	89	6	1
14M	Mekelle	100	30	39	89	46	1
15M	Mekelle	120	40	39	89	6	1
16M	Mekelle	140	50	39	89	46	1
17M	Mekelle	160	30	39	89	46	1
19M	Mekelle	50	38	39	89	6	1
21M	Mekelle	100	50	39	89	46	1
22M	Mekelle	25	24	39	89	6	1
24M	Mekelle	120	30	39	89	46	1
25M	Mekelle	140	35	39	89	46	1
27M	Mekelle	160	37	39	89	6	1
31M	Mekelle	25	20	39	89	46	1
32M	Mekelle	140	45	39	89	6	1
35M	Mekelle	160	35	39	89	46	1
36M	Mekelle	50	30	39	89	6	1
37M	Mekelle	100	40	39	89	6	1
39M	Mekelle	120	50	39	89	46	1
40M	Mekelle	50	32	39	89	46	1
44M	Mekelle	160	40	39	89	6	1
45M	Mekelle	160	50	39	89	46	1
46M	Mekelle	25	22	39	89	6	1
49M	Mekelle	100	30	39	89	46	1
52M	Mekelle	120	40	39	89	6	1
53M	Mekelle	140	50	39	89	46	1
54M	Mekelle	160	30	39	89	46	1
47M	Mekelle	50	38	39	89	6	1
58M	Mekelle	100	50	39	89	46	1
59M	Mekelle	25	24	39	89	6	1
64M	Mekelle	120	30	39	89	46	1
65M	Mekelle	140	35	39	89	46	1
73M	Mekelle	160	37	39	89	6	1
74M	Mekelle	25	21.9	39	89	46	1
77M	Mekelle	120	45.8	39	89	6	1
78M	Mekelle	120	45.1	39	89	46	1
88M	Mekelle	50	45.2	39	89	6	1

91M	Mekelle	50	41.4	39	89	46	1
92M	Mekelle	100	35.5	39	89	6	1
93M	Mekelle	140	30	39	89	46	1
94M	Mekelle	25	23	39	89	6	1
95M	Mekelle	100	43.3	39	89	6	1
96M	Mekelle	50	30	39	89	6	1
98M	Mekelle	120	50	39	89	46	1
99M	Mekelle	160	45.5	39	89	46	1
100M	Mekelle	120	35	39	89	46	1
84M	Mekelle	50	41.4	39	89	46	1
90M	Mekelle	160	45.5	39	89	46	1
34M	Mekelle	50	45.2	39	89	6	1
69M	Mekelle	25	21.9	39	89	46	1
82M	Mekelle	100	43.3	39	89	6	1
56M	Mekelle	140	33.9	39	89	46	1
57M	Mekelle	140	40	39	89	46	1
70M	Mekelle	120	45.1	39	89	46	1
76M	Mekelle	100	45	39	89	6	1
86M	Mekelle	140	40	39	89	46	1
87M	Mekelle	140	33.9	39	89	6	1
81M	Mekelle	25	23	39	89	6	1
72M	Mekelle	120	35	39	89	46	1
62M	Mekelle	100	45	39	89	46	1
68M	Mekelle	100	35.5	39	89	6	1
75M	Mekelle	140	30	39	89	46	1
61M	Mekelle	120	45.8	39	89	6	1
71M	Mekelle	140	45	39	89	6	1
89M	Mekelle	160	35	39	89	46	1

PH = Plant Height, SL = Stem Length, FC = Flower Color, LS = Leaf Shape, LA = Leaf Arrangement and PGH = Plant Growth Habit

Appendix 3: List of individuals with their belonging cluster No.

Individuals	PH	SL	LS	LA	FC	PGH	Cluster classification
29G	80	58.5	39	89	48	1	Cluster III
66G	120	55	39	89	9	1	Cluster VI
87G	150	62.8	39	89	48	1	Cluster V
74G	180	65	39	89	9	1	Cluster III
88G	120	60	39	89	48	1	Cluster III
15G	80	52	39	89	48	1	Cluster II
3G	80	50	39	89	9	1	Cluster II
54G	150	70	39	89	9	1	Cluster III
10G	180	70	39	89	48	1	Cluster VI
46G	120	65	39	89	9	1	Cluster III
22G	200	75.5	39	89	48	1	Cluster VI
57G	200	60	39	89	9	1	Cluster III
65G	150	55	39	89	48	1	Cluster II
4G	80	62	39	89	9	1	Cluster V
61G	180	75.3	39	89	9	1	Cluster II
19G	80	65	39	89	48	1	Cluster VI
39G	120	70	39	89	48	1	Cluster II
84G	180	61	39	89	9	1	Cluster III
76G	150	78	39	89	48	1	Cluster II
45G	120	50	39	89	9	1	Cluster II
50G	80	50	39	89	9	1	Cluster V
38G	80	52	39	89	48	1	Cluster II
34G	120	60	39	89	48	1	Cluster VI
59G	180	65	39	89	9	1	Cluster II
21G	150	62.8	39	89	48	1	Cluster III
27G	120	55	39	89	9	1	Cluster III
36G	80	58.5	39	89	48	1	Cluster VI
13G	150	70	39	89	9	1	Cluster II
48G	180	70	39	89	48	1	Cluster V
71G	120	65	39	89	9	1	Cluster III
64G	200	75	39	89	48	1	Cluster II
47G	200	60	39	89	9	1	Cluster III
40G	150	55	39	89	48	1	Cluster VI
100G	80	62	39	89	9	1	Cluster V
98G	180	75	39	89	9	1	Cluster VI
12G	80	65	39	89	48	1	Cluster III
8G	120	70	39	89	48	1	Cluster III
42G	180	61	39	89	9	1	Cluster V
35G	150	78	39	89	48	1	Cluster II
18G	120	50	39	89	9	1	Cluster III
96G	200	65	39	89	48	1	Cluster III
5G	150	80	39	89	9	1	Cluster III
20G	200	50	39	89	9	1	Cluster III
86G	180	55	39	89	48	1	Cluster VI

79G	180	51.5	39	89	9	1	Cluster V
4G	120	80	39	89	48	1	Cluster V
56G	80	70	39	89	9	1	Cluster III
78G	200	80	39	89	9	1	Cluster II
85G	200	55	39	89	48	1	Cluster VI
80G	200	70	39	89	9	1	Cluster III
99G	150	50	39	89	48	1	Cluster II
91G	180	80	39	89	9	1	Cluster VI
25G	120	75	39	89	9	1	Cluster V
14G	80	72	39	89	48	1	Cluster II
92G	200	55	39	89	48	1	Cluster III
52G	200	65	39	89	48	1	Cluster V
30G	120	80	39	89	48	1	Cluster VI
58G	200	50	39	89	9	1	Cluster V
77G	80	70	39	89	9	1	Cluster III
9G	180	55	39	89	48	1	Cluster II
82G	150	50	39	89	48	1	Cluster III
69G	200	70	39	89	9	1	Cluster III
1G	180	51.5	39	89	9	1	Cluster II
16G	200	80	39	89	9	1	Cluster VI
89G	150	80	39	89	9	1	Cluster III
3M	25	20	39	89	46	1	Cluster IV
4M	100	40	39	89	6	1	Cluster I
7M	50	32	39	89	46	1	Cluster I
9M	160	40	39	89	6	1	Cluster VI
12M	160	50	39	89	46	1	Cluster VI
13M	25	22	39	89	6	1	Cluster VI
14M	100	30	39	89	46	1	Cluster I
15M	120	40	39	89	6	1	Cluster VI
16M	140	50	39	89	46	1	Cluster IV
17M	160	30	39	89	46	1	Cluster IV
19M	50	38	39	89	6	1	Cluster VI
21M	100	50	39	89	46	1	Cluster IV
22M	25	24	39	89	6	1	Cluster IV
24M	120	30	39	89	46	1	Cluster IV
25M	140	35	39	89	46	1	Cluster VI
27M	160	37	39	89	6	1	Cluster I
31M	25	20	39	89	46	1	Cluster I
32M	140	45	39	89	6	1	Cluster I
35M	160	35	39	89	46	1	Cluster I
36M	50	30	39	89	6	1	Cluster VI
37M	100	40	39	89	6	1	Cluster IV
39M	120	50	39	89	46	1	Cluster VI
40M	50	32	39	89	46	1	Cluster VI
44M	160	40	39	89	6	1	Cluster VI
45M	160	50	39	89	46	1	Cluster I

46M	25	22	39	89	6	1	Cluster I
49M	100	30	39	89	46	1	Cluster VI
52M	120	40	39	89	6	1	Cluster IV
53M	140	50	39	89	46	1	Cluster VI
54M	160	30	39	89	46	1	Cluster I
47M	50	38	39	89	6	1	Cluster I
58M	100	50	39	89	46	1	Cluster I
59M	25	24	39	89	6	1	Cluster I
64M	120	30	39	89	46	1	Cluster IV
65M	140	35	39	89	46	1	Cluster IV
73M	160	37	39	89	6	1	Cluster V
74M	25	21.9	39	89	46	1	Cluster VI
77M	120	45.8	39	89	6	1	Cluster IV
78M	120	45.1	39	89	46	1	Cluster I
88M	50	45.2	39	89	6	1	Cluster I
91M	50	41.4	39	89	46	1	Cluster I
92M	100	35.5	39	89	6	1	Cluster I
93M	140	30	39	89	46	1	Cluster VI
94M	25	23	39	89	6	1	Cluster VI
95M	100	43.3	39	89	6	1	Cluster VI
96M	50	30	39	89	6	1	Cluster VI
98M	120	50	39	89	46	1	Cluster V
99M	160	45.5	39	89	46	1	Cluster IV
100M	120	35	39	89	46	1	Cluster VI
84M	50	41.4	39	89	46	1	Cluster IV
90M	160	45.5	39	89	46	1	Cluster V
34M	50	45.2	39	89	6	1	Cluster II
69M	25	21.9	39	89	46	1	Cluster VI
82M	100	43.3	39	89	6	1	Cluster IV
56M	140	33.9	39	89	46	1	Cluster IV
57M	140	40	39	89	46	1	Cluster VI
70M	120	45.1	39	89	46	1	Cluster VI
76M	100	45	39	89	6	1	Cluster I
86M	140	40	39	89	46	1	Cluster V
87M	140	33.9	39	89	6	1	Cluster V
81M	25	23	39	89	6	1	Cluster IV
72M	120	35	39	89	46	1	Cluster IV
62M	100	45	39	89	46	1	Cluster IV
68M	100	35.5	39	89	6	1	Cluster IV
75M	140	30	39	89	46	1	Cluster IV
61M	120	45.8	39	89	6	1	Cluster IV
71M	140	45	39	89	6	1	Cluster I
89M	160	35	39	89	46	1	Cluster VI

PH = Plant Height, SL = Stem Length, FC = Flower Color, LS = Leaf Shape, LA = Leaf Arrangement and PGH = Plant Growth Habit

Appendix 4: List of Primers

No.	Sequences (5'→3')	No.	Sequences (5'→3')
1	$(GA)_9C$	9	$(TC)_8C$
2	$(GT)_9C$	10	$(TC)_8G$
3	$(GT)_9T$	11	$(AC)_8G$
4	**$(GA)_8C$**	12	**$(TG)_8A$**
5	$(CT)_8G$	13	**$(AC)_8C$**
6	$(AG)_8C$	14	**$(ATC)_6T$**
7	$(AG)_8G$	15	$(ATC)_6C$
8	**$(GA)_8A$**	16	$(ATG)_6G$

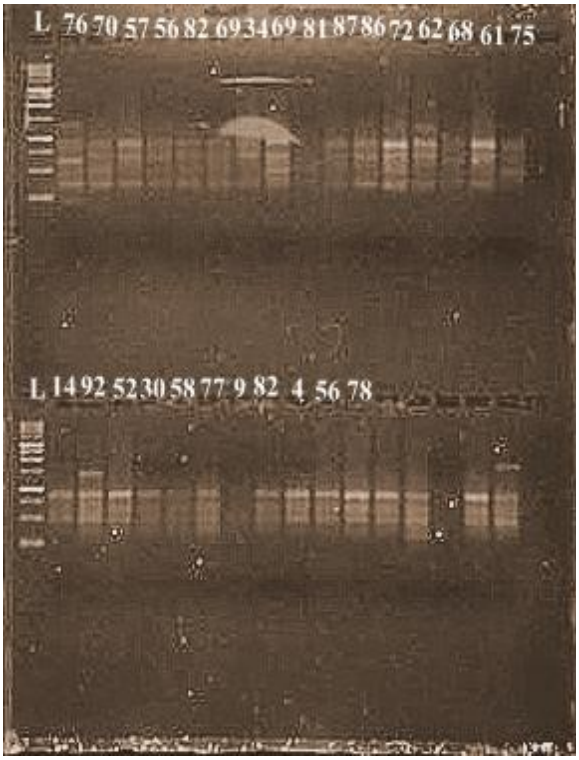
Appendix 5: (A) Morphological characterization of *Urtica simensis* in Mekelle
(B) Morphological characterization of *Urtica simensis* in Gondar

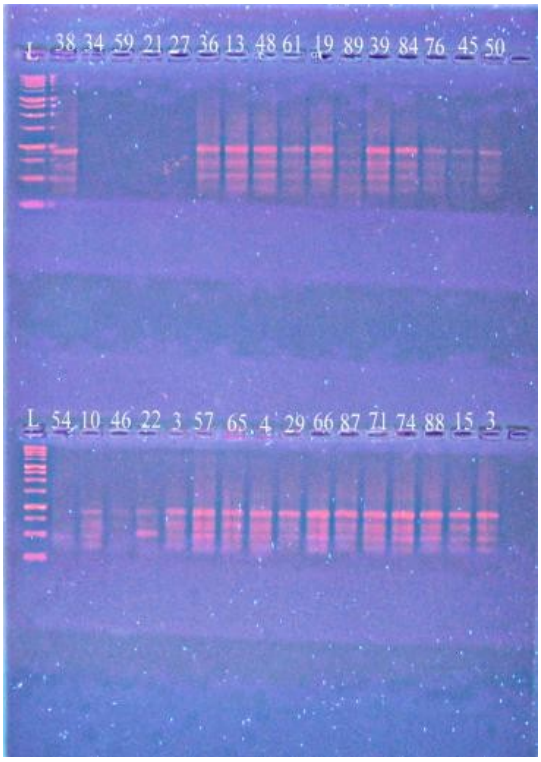
A. Morphological characterization of *Urtica simensis* in Gondar

B. Morphological characterization of *Urtica simensis* in Mekelle

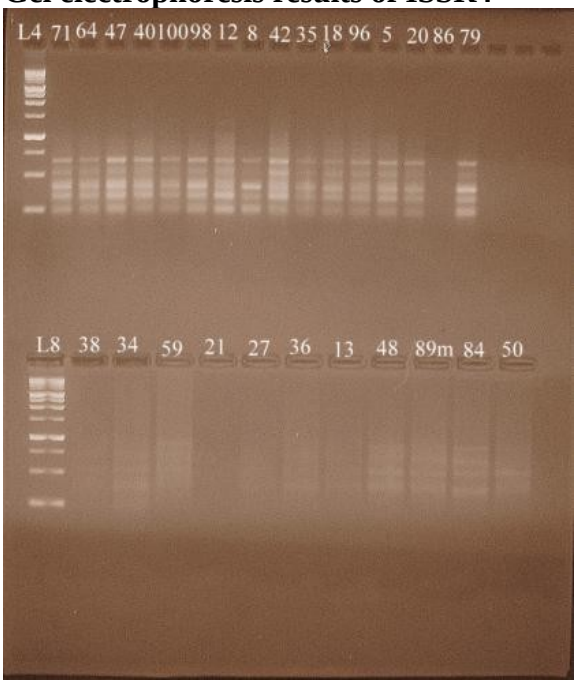
Appendix 6: ISSR gel electrophoresis data representatives

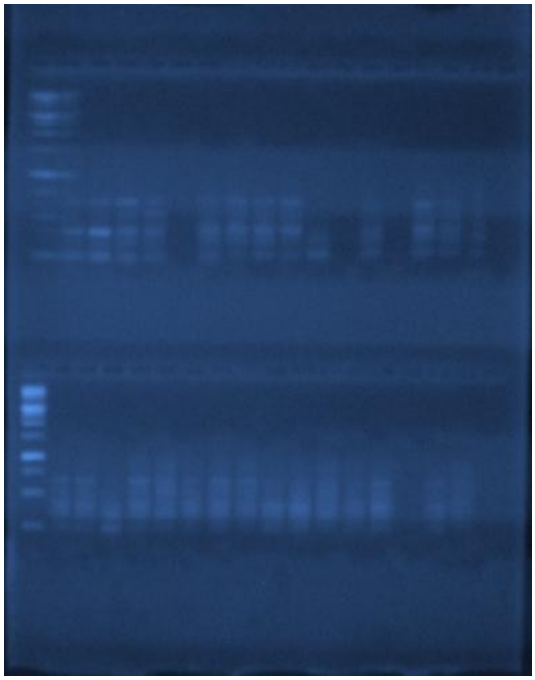
Gel electrophoresis result of ISSR12



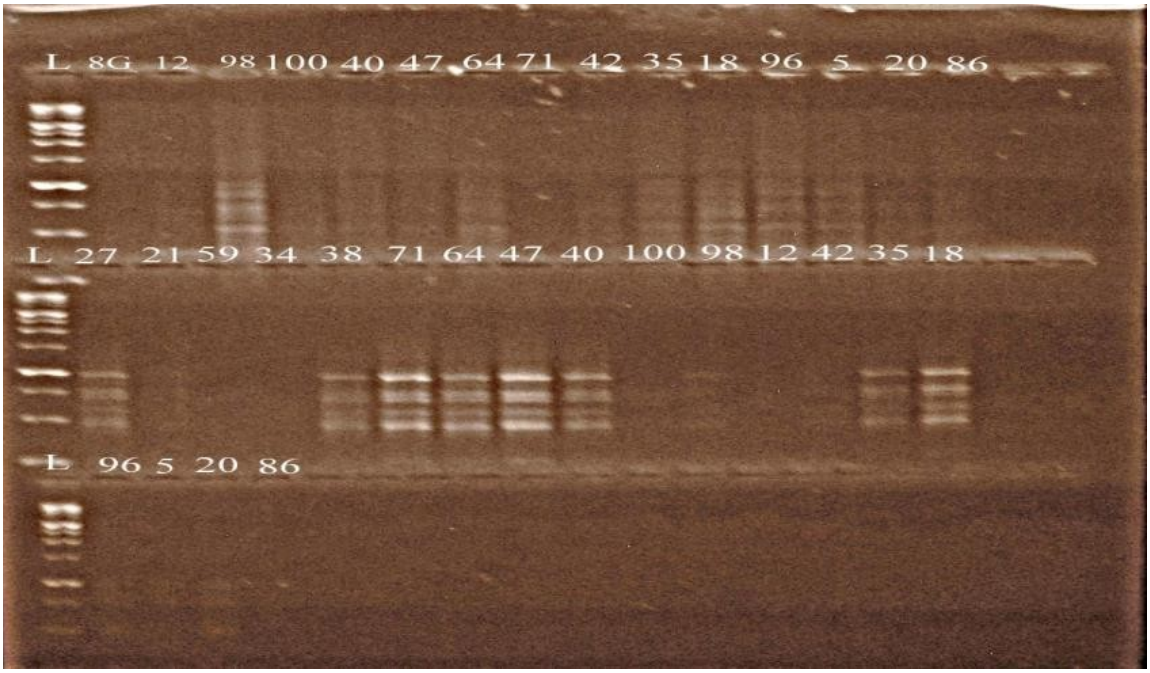


Gel electrophoresis results of ISSR4





Gel electrophoresis results ISSR13 and ISSR8



Gel electrophoresis results of ISSR14