GENETIC VARIATION AMONG Stevia rebaudiana Bertoni GENOTYPES UNDER LIGHT TREATMENT ON MORPHO-AGRONOMIC TRAITS AND ISSR MOLECULAR MARKERS

DHULFIQAR AAMER ABDULAMEER

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ISSR MOLECULAR MARKERS

By

DHULFIQAR AAMER ABDULAMEER

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfillment of the Requirements for the Degree of Master of Science

February 2018
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DEDICATION

To my lovely country Iraq

To my beloved family who always supported and kept praying for me day and night
to be what I am now

And

To my friends who boosted me all these past years
Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Science

GENETIC VARIATION AMONG Stevia rebaudiana Bertoni GENOTYPES UNDER LIGHT TREATMENT ON MORPHO-AGRONOMIC TRAITS AND ISSR MOLECULAR MARKERS

By

DHULFIQAR AAMER ABDULAMEER

February 2018

Chairman : Professor Mohd Rafii Yusop, PhD
Faculty : Agriculture

Stevia rebaudiana Bertoni is a perennial herb with natural sweetening compounds that are 230 times sweeter than sucrose with no calorific value. It belongs to the family Asteraceae (Compositae) and is native to Paraguay. It is a good source of carbohydrates, protein, crude fibers and minerals. Stevioside and rebaudioside A are the two major sweetening compounds. Stevia is a short day plant which form flowers when day length is less than 12 hours and it is resulting in low biomass production. In this study, a set of stevia genotypes collected from different sources were evaluated for yield components and flowering characteristics both under short day length (12 hours) and long day length (14 hours) environments. Genetic variation among those genotypes was also investigated using ISSR molecular markers. A total of twenty-six stevia genotypes including two hybrids were collected. The propagation of all collected stevia genotypes was achieved through stem micro-cutting within six months. Eight genotypes namely, CHV1, Morita2, MS012, MS012 (4x), SA18, TPU, and hybrids IIUM-F1 and UiTM-F1 which had shown good propagation ability under mist-chamber propagation boxes and were evaluated for the agronomic performance under short day length (12 hours) and long day length (14 hours) environments using a split plot design. In general, all the agronomic characteristics of pooled genotypes were increased significantly under the extended light (14 hours). Stevia plants under sunlight (12 hours) started to flowering after 52 days after transplanted. On the other hand stevia plants under extended light were not flowered up to 134 days at harvesting stage. Combined biomass fresh weight mean of the eight genotypes was increased 728% under extended light compared to genotypes kept under sunlight. The biomass fresh weight mean and plant height of genotype SA18 was the highest in both extended and sunlight conditions (35.82 g and 3.96 g; 92.47 cm and 24.92 cm respectively). The Pearson correlation coefficient showed that biomass fresh weight had strongly positively correlated with all the
characters except number of branches. Genetic variation and cluster analysis based on ISSR markers showed that genotypes CHV1 and H2 were the most closely related genotypes with the highest similarity index (54%). On the other hand, genotypes MS012 and TPU were the distantly related genotypes with the lowest similarity index (24%). In conclusion, stevia plants kept under extended light showed better performance than under sunlight. Three genotypes, SA18, TPU and CHV1 were shown better performance than other genotypes, and are recommended for further utilization in future stevia breeding programs.
Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

VARIASI GENETIK KALANGAN GENOTIP Stevia rebaudiana Bertoni DI BAWAH RAWATAN CAHAYA KE ATAS CIRI MORFO-AGRONOMI DAN PENANDA MOLEKUL ISSR

Oleh

DHULFIQAR AAMER ABDULAMEER

Februari 2018

Pengerusi : Profesor Mohd Rafii Yusop, PhD
Fakulti : Pertanian

Stevia rebaudiana Bertoni adalah herba saka yang mengandungi kompau pemanis semulajadi sebanyak 230 kali lebih manis daripada sukrosa dan ianya tanpa nilai kalori. Ia tergolong di bawah keluarga Asteraceae (Compositae) dan yang berasal dari Paraguay. Ia merupakan sumber karbohidrat, protein, fiber mentah dan zat galian. Stevioside dan rebaudioside A adalah dua kompau pemanis utama spesies ini. Stevia adalah tumbuhan hari-pendek yang mengeluarkan bunga apabila waktu siang kurang dari 12 jam dan seterusnya ianya mengakibatkan pengeluaran biojisim yang rendah. Dalam kajian ini, satu kumpulan genotip stevia yang dikumpulkan dari beberapa sumber yang berbeza telah dinilai dari segi komponen hasil dan ciri pembungaan di bawah persekitaran jangka siang yang pendek (12 jam) dan jangka siang yang panjang (14 jam). Variasi genetik di kalangan genotip juga telah dikaji dengan menggunakan penanda molikul ISSR. Sejumlah dua puluh enam aksesi stevia termasuk dua hibrid telah dikumpulkan. Pembiakan semua aksesi stevia telah dijalankan melalui keratan mikro batang dalam tempoh enam bulan. Lapan genotip iaitu; CHV1, Morita2, MS012, MS012 (4x), SA18, TPU, dan hibrid IIUM-F1 dan UiTM-F1), yang menunjukkan prestasi pembiakan yang baik di bawah kotak pembiakan berkabus, dan telah dinilai dari segi prestasi agronomi di bawah jangka siang yang pendek (12 jam) dan jangka siang yang panjang (14 jam) dalam satu eksperimen faktorial. Secara umumnya, kesemua ciri agronomi bagi kombinasi genotip didapati bertambah di bawah pencahayaan yang dilanjutkan. Pokok stevia di bawah rawatan cahaya (12 jam) mulai berbunga 52 hari selepas penanaman. Sebaliknya pokok di bawah pencahayaan lanjutan, pokok stevia tidak berbunga sehingga 134 hari selepas penanaman pada peringkat penuaan. Berat basah biomass keseluruhan bagi lapan genotip tersebut telah meningkat 728% di bawah pencahayaan lanjutan berbanding pokok di bawah cahaya matahari. Purata berat basah biomass dan ketinggian pokok aksesi SA18 didapati adalah tertinggi di bawah
kedua-dua persekitaran, pencahayaan lanjutan dan cahaya matahari (masing-masing 135.82 g dan 3.96 g; 92.47 cm dan 24.92 cm). Pekali korelasi Pearson menunjukkan berat biomass segar adalah berkorelasi tinggi secara positif dengan semua ciri-ciri kecuali dengan bilangan dahan. Analisa variasi genetik dan kluster berdasarkan penanda ISSR menunjukkan genotip CHV1 and H2 mempunyai perkaitan genotip yang paling rapat dengan indeks kesamaan tertinggi (54%). Sebaliknya, genotip MS012 and TPU adalah genotip yang paling berbeza dengan indeks kesamaan paling rendah (24%). Kesimpulannya, pokok di bawah pencahayaan lanjutan menunjukkan prestasi yang lebih baik daripada pencahayaan matahari. Tiga genotip iaitu SA18, TPU dan CHV1 didapati berprestasi lebih baik daripada semua genotip lain, dan dicadangkan untuk digunakan seterusnya di dalam program pembiakbakaan stevia di masa hadapan.
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*Dhulfiqar Aamer Abdulameer, 2018*
I certify that a Thesis Examination Committee has met on 13 July 2018 to conduct the final examination of Dhulfiqar Aamer Abdulameer on his thesis entitled "Genetic Variation Among Stevia rebaudiana Bertoni Genotypes Under Light Treatment on Morpho-Agronomic Traits and ISSR Molecular Markers" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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Name of Member of Supervisory Committee: Associate Professor Dr. Zulkefly Sulaiman
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Title</th>
<th>Pages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>LITERATURE REVIEW</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2.1 Taxonomy and Origin</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2.2 Classification of Stevia</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>2.3 Genus of Stevia</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2.4 Geographic range and habitat of stevia</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2.5 Glycosides and Chemical Constituents</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2.6 Cyto genetic</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>2.7 Morphology</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.8 Flower structure</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>2.9 Seed</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2.10 Utilization of Stevia</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2.11 Stevia Product and Commercialization</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>2.12 Chemical Compounds in Stevia</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>2.13 Cultivation</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.14 Propagation</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.14.1 Environmental Factors</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.14.2 Methods of Propagation</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>2.14.2.1 Stem Cutting Propagation</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.15 Stevia breeding</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.15.1 Stevia breeding in the world</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>2.15.2 Stevia Breeding in Malaysia</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>2.16 Genetic Diversity</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2.16.1 Morpho Agronomic Diversity</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>2.16.2 Genetic and Metabolic Diversity Using RAPD and HPLC</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>2.17 Extended Day Length</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>2.17.1 Day Length</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>2.17.2 Light</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>2.17.3 Photoperidism</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>2.17.4 Effect of Photoperiods on Growth and Development</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>2.18 PCR-Based Markers</td>
<td>21</td>
</tr>
<tr>
<td></td>
<td>2.18.1 Inter-Single Simple Repeat (ISSR) marker</td>
<td>21</td>
</tr>
</tbody>
</table>
## MATERIALS AND METHODS

### 3.1 Stevia genotypes

### 3.2 Propagation of stevia through stem micro-cuttings
- **3.2.1 Propagation Box**
- **3.2.2 Acclimatization of stevia plantlets**
- **3.2.3 Selection of genotypes based on number of plantlets produced**

### 3.3 Evaluation of the agronomic performance of selected stevia genotypes
- **3.3.1 Lighting treatments**
- **3.3.2 Experimental site and design**
- **3.3.3 Parameters evaluated**
- **3.3.4 Statistical analysis**

### 3.4 Molecular diversity among stevia genotypes
- **3.4.1 Plant Materials**
- **3.4.2 Samples preparation**
- **3.4.3 DNA extraction and quantification**
- **3.4.4 Selection and validation of ISSR primers**
- **3.4.5 PCR optimization**
- **3.4.6 Analysis of polymorphism through gel electrophoresis**
- **3.4.7 Analysis of genetic relationship between stevia genotypes**

## RESULTS AND DISCUSSION

### 4.1 Propagation and selection of stevia genotypes based on stem micro-cuttings performance

### 4.2 Evaluating the agronomic performance of selected stevia genotypes

### 4.3 Molecular diversity of stevia genotypes
- **4.3.1 ISSR makers screening**
- **4.3.2 Genetic variation among stevia genotypes**
- **4.3.3 Polymorphism information content (PIC) value**

## CONCLUSION AND RECOMMENDATIONS FOR FUTURE STUDIES

### 5.1 Conclusion

### 5.2 Recommendations for future studies

## REFERENCES

## APPENDICES

## BIODATA OF STUDENT

## PUBLICATION
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Ethnomedical use of stevia</td>
</tr>
<tr>
<td>2.2</td>
<td>Commercially obtainable stevia products in USA market</td>
</tr>
<tr>
<td>2.3</td>
<td>Amount of sweet glycoside in <em>Stevia rebaudiana</em> leaves (% of the leaves dry weight)</td>
</tr>
<tr>
<td>2.4</td>
<td>List of stevia genotypes developed</td>
</tr>
<tr>
<td>2.5</td>
<td>Stevia germplasm available at the International Islamic University of Malaysia (IIUM)</td>
</tr>
<tr>
<td>2.6</td>
<td>List of stevia collected by MARDI from other countries</td>
</tr>
<tr>
<td>2.7</td>
<td>Stevia genotypes and area of collection in Malaysia</td>
</tr>
<tr>
<td>3.1</td>
<td>List of collected stevia genotypes</td>
</tr>
<tr>
<td>3.2</td>
<td>Eleven ISSR primers used for genetic diversity of stevia genotypes</td>
</tr>
<tr>
<td>3.3</td>
<td>ISSR primers used for analysis of <em>Stevia rebaudiana</em> Bertoni genotypes with their melting and annealing temperature</td>
</tr>
<tr>
<td>4.1</td>
<td>Number of propagated plants for each stevia genotype</td>
</tr>
<tr>
<td>4.2</td>
<td>Percentage of relative efficiency between extended light and sunlight of pooled genotypes on the quantitative traits</td>
</tr>
<tr>
<td>4.3</td>
<td>Percentage of relative efficiency between extended light and sunlight of different genotypes on the quantitative traits</td>
</tr>
<tr>
<td>4.4</td>
<td>Analysis of variance on agronomic traits in stevia genotypes</td>
</tr>
<tr>
<td>4.5</td>
<td>Mean comparison (Means± S.D) on agronomic traits in stevia genotypes under sunlight and extended light conditions</td>
</tr>
<tr>
<td>4.6</td>
<td>Pearson Correlation coefficients among quantitative traits of stevia genotypes pooled data under lightning conditions</td>
</tr>
<tr>
<td>4.7</td>
<td>Principal components analysis (PCA) for 8 morphological traits among the eight stevia genotypes</td>
</tr>
<tr>
<td>4.8</td>
<td>ISSR polymorphic primers, number of polymorphic loci, percentage of polymorphic loci, and estimates of variability across eight stevia genotypes</td>
</tr>
</tbody>
</table>
4.9 Genetic similarities among *Stevia rebaudiana* Bertoni genotypes based on eight ISSR molecular markers

xiii
### LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1</td>
<td>Three genotypes (MS012, MS007 and MS028) with promising characteristics under Malaysia's condition</td>
<td>15</td>
</tr>
<tr>
<td>3.1</td>
<td>Propagation steps for 26 stevia genotypes using mist-chamber propagation box</td>
<td>25</td>
</tr>
<tr>
<td>4.1</td>
<td>Dendogram using average linkage among the selected eight stevia genotypes</td>
<td>42</td>
</tr>
<tr>
<td>4.2</td>
<td>Scatter plot of PC1 and PC2 of eight stevia genotypes</td>
<td>43</td>
</tr>
<tr>
<td>4.3</td>
<td>Banding patterns of IS57 primer; L: 100 base pair ladder, 1: CHV1, 2: SA18, 3: MS012, 4: 4X, 5: F1, 6: TPU, 7: H2 and 8: Morita</td>
<td>46</td>
</tr>
<tr>
<td>4.4</td>
<td>Banding patterns of IS20 primer; L: 100 base pair ladder, 1: CHV1, 2: SA18, 3: MS012, 4: 4X, 5: F1, 6: TPU, 7: H2 and 8: Morita</td>
<td>46</td>
</tr>
<tr>
<td>4.5</td>
<td>Banding patterns of IS21 primer; L: 100 base pair ladder, 1: CHV1, 2: SA18, 3: MS012, 4: 4X, 5: F1, 6: TPU, 7: H2 and 8: Morita</td>
<td>47</td>
</tr>
<tr>
<td>4.6</td>
<td>Banding patterns of IS20 primer; L: 100 base pair ladder, 1: CHV1, 2: SA18, 3: MS012, 4: 4X, 5: F1, 6: TPU, 7: H2 and 8: Morita</td>
<td>47</td>
</tr>
<tr>
<td>4.7</td>
<td>Banding patterns of IS56 primer; L: 100 base pair ladder, 1: CHV1, 2: SA18, 3: MS012, 4: 4X, 5: F1, 6: TPU, 7: H2 and 8: Morita</td>
<td>48</td>
</tr>
<tr>
<td>4.8</td>
<td>Banding patterns of IS31 primer; L: 100 base pair ladder, 1: CHV1, 2: SA18, 3: MS012, 4: 4X, 5: F1, 6: TPU, 7: H2 and 8: Morita</td>
<td>48</td>
</tr>
<tr>
<td>4.9</td>
<td>Banding patterns of IS16 primer; L: 100 base pair ladder, 1: CHV1, 2: SA18, 3: MS012, 4: 4X, 5: F1, 6: TPU, 7: H2 and 8: Morita</td>
<td>49</td>
</tr>
<tr>
<td>4.10</td>
<td>Banding patterns of IS85 primer; L: 100 base pair ladder, 1: CHV1, 2: SA18, 3: MS012, 4: 4X, 5: F1, 6: TPU, 7: H2 and 8: Morita</td>
<td>49</td>
</tr>
<tr>
<td>4.11</td>
<td>Dendogram illustrating genetic relationship among eight stevia genotypes, produced by UPGMA cluster tree analysis (NTSYS pc) calculated from 86 ISSR markers generated from eight ISSR primer</td>
<td>52</td>
</tr>
</tbody>
</table>
# LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AFLP</td>
<td>Amplified fragment length polymorphisms</td>
</tr>
<tr>
<td>bp</td>
<td>Base pair</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>ISSR</td>
<td>Inter simple sequence repeat</td>
</tr>
<tr>
<td>NAA</td>
<td>α- naphthaleneacetic acid</td>
</tr>
<tr>
<td>NTSYS-pc</td>
<td>Numerical taxonomy and multivariate analysis system</td>
</tr>
<tr>
<td>PCA</td>
<td>Principal component analysis</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase Chain Reactions</td>
</tr>
<tr>
<td>POPGENE</td>
<td>Population genetic analysis</td>
</tr>
<tr>
<td>RAPD</td>
<td>Random amplified polymorphic DNA</td>
</tr>
<tr>
<td>SSR</td>
<td>Simple sequence repeat</td>
</tr>
<tr>
<td>Taq</td>
<td>Thermus aquaticus</td>
</tr>
<tr>
<td>TBE</td>
<td>Tris-borate EDTA</td>
</tr>
<tr>
<td>UPGMA</td>
<td>Unweighted pair group method based on arithmetic averages</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

Human interest in natural products is increasing day by day. *Stevia rebaudiana* Bertoni commonly known as candy leaf or sweet leaf (generically as stevia) is an ancient perennial shrub of South America. It belongs to the same family with sunflower (*Asteraceae*) and produces diterpene glycosides that are 300 times lower in calorie sweeteners than saccharose. About 240 stevia species have been identified and characterized, among these species the sweetest essences have successfully been extracted from *Stevia rebaudiana*. Presently, stevia has a great potential as a modern agricultural crop since consumer demand increased for herbal foods, zero-calorie sweetener and appropriate gluco-regulation for consumers afflicted with diabetes and prevention of dental cavities (Thomas, 2010).

The chemical compounds that produce sweetness in stevia are various steviol glycosides while the major components are stevioside and rebaudioside A (Singh and Rao, 2005). Stevioside is the sweet glycoside with the highest concentration found in stevia and contributing about 60-70% of total glycosides (Yadav *et al.*, 2011) but it has licorice aftertaste. However, rebaudioside A contributing about 30-40% of total glycosides in stevia and has no aftertaste (Midmore and Rank, 2002). In addition to sugary compounds, stevia extracts also contain a high level of therapeutic properties that plays an imperative role as antioxidant, antimicrobial, antifungal activities and also serves a good source of fiber and minerals (Abou-Arab *et al.*, 2010).

In Malaysia, stevia was introduced in the 1970s. The initial studies on propagation, cultivation and germplasm evaluation were carried out by Tan *et al.* (2008) under Malaysian Agriculture Research and Development Institute (MARDI). From the study, out of 69 genotypes five genotypes were recommended for local cultivation. However, the major problem for stevia cultivation in Malaysia is photoperiod sensitiveness that affects the early flowering which contributes to low leaf yield and stunted plant growth. Several reports from researchers from Universiti Teknologi MARA (UiTM), Universiti Putra Malaysia (UPM) and International Islamic Universiti Malaysia (IIUM) found that no suitable variety for high biomass production in Malaysia (per comm. Osman 2018). Meanwhile, other countries such as China and Japan, are successful in development of suitable stevia varieties for their local cultivation.

Genetically, *S. rebaudiana* B. is a diploid plant with 22 chromosomes. Several species of stevia have similar characteristics but possess different karyotypes caused by the gain or loss of chromatin due to spontaneous mutation (Frederico and Ruas, 1996). Polyploidy is also recorded in *S. rebaudiana* B. through artificial induction by developing lines containing elevated levels of stevioside (Oliveira *et al.*, 2004). The
stevia has been extensively documented as self-incompatible and pollination assisted by insects (Ramesh et al., 2006). Goettemoeller and Ching (1999) reported that fertile seeds were obtained from self-pollination, however, how much seeds collected was unknown and neither was there any verification of the progeny testing. As a whole, stevia is a highly outcrossing plant that is challenging for breeders to develop inbred lines, whereas, high heterozygosity offers variation to develop hybrid variety.

The agronomical practices for cultivation of stevia needs an optimum temperature of 20-28 °C for good growth, clay sandy soil that rich in organic fertilizers with intensive damping, high illumination and the day length of more than 13 hours per day (Soejarto et al., 1982). The local environment condition of Malaysia can provide the plant with all these conditions except the required day length which leads to early flowering. Because the harvesting of stevia aerial parts need to be carried out before flowering as the level of the sweetness decreases as soon as the plants begin to flower. In addition, the production of stevia varieties with a longer vegetative period in the tropical environment requires a depth study on genetic diversity among the available genotypes.

This study was designed with a set of stevia genotypes collected from different sources evaluated for agronomic traits under short day length (<13 hours) and long day length (>13 hours) conditions. The genetic variation among these genotypes was determined using agronomic characteristics and ISSR molecular markers with the following objectives;

i. To evaluate the agronomic performance of stevia genotypes collected from different sources under short and long day length environmental conditions.
ii. To assess the genetic diversity among stevia genotypes using ISSR molecular markers.
iii. To identify superior genotypes for future stevia breeding programs.
REFERENCES


