DEDICATION

To my late parents,

My brothers, sisters,

My daughter, son

and my wife

With gratitude and

appreciation

Salah Eldein

Acknowledgement

I wish to express my deep thanks and gratitude to Professor A. Aziz Makkawi who acted as my supervisor and Iam also indebted to Dr. Mohamed Kair Abdalla Ahmed who acted as my co-supervisor in their study for his valuable suggestions, advice and guidance.

I wish to express my sincere gratitude to Dr. Galal M. Yousif and Dr. Ibtisam I. Mekki for their keen enthusiasm, guidance, most valuable assistance, advice and encouragement throughout the study.

My thanks are extended to Prof. Haniya A. El-Itriby, Director of Agricultural Genetic Engineering Research Institute (AGERI), Agricultural Research Center (ARC), Egypt and Dr. Sami S. Adwy (AGERI)(ARC), for their close supervision, providing materials, useful information, endless help and unlimited support to carryout this work.

I would like to thank all members of AGERI, especially staff members of Molecular Genetics and Genome Mapping Lab (MGGM).

Iam also grateful to the Egyptian Government (Ministry of Agriculture) for granting me the scholarship to complete this study.

My thanks are also extended to the Central Research Committee, of the Sudan University of Science and Technology for funding part of this study.

I wish to express my sincere gratitude to all members of the College of Medical and Science Laboratory Technology (SUST), and National Health Laboratory (Ministry of Health), for their technical assistance and continuous encouragement throughout this work.

Grateful thanks are due to the Ministry of Science and Technology – Animal Production Research Center at Kuku, Atbara and Um-Benain for providing blood samples of the different breeds.

Sincere thanks are due to my colleagues and staff members of the Animal Production Department, College of Agricultural Studies, Sudan University of Science and Technology.

Lastly my grateful thanks are due to my family for support and for showing great patience during this work.

ABSTRACT

Two different types of molecular markers (RAPD and AFLP) were used to measure genetic diversity and fingerprint 4 local breeds of Sudanese cattle namely Western Baggara (Nialawy), Nilotic (Majock), Butana and Kenana.

The level of polymorphism revealed by RAPD and AFLP among the four breeds was 51.5% and 91.5% respectively. While RAPD techniques recorded 22.9%, 36.8%, 24.4% and 41.4% within the 4 local breeds, respectively.

Only 8 out of 22 primers (36%) used in the RAPD analysis and 11 primers combinations used in the AFLP techniques gave unique markers that singularly identified specific breeds.

The RAPD and AFLP data matrix was utilized to estimate the genetic similarity using Jaccard's coefficient. It ranged from 78.4% to 84.8% with an average of 81.6% and from 46.9 to 80.3% with an average of 63.6% respectively. While it ranged from 98.9% to 92.9% with an average of 95.9%; from 96.3% to 88.2% with an average of 92.2%, from 98.7% to 88% with an average of 93.3% and from 97.2% to 80.3% with an average of 88.7% within the above four local breeds, respectively using RAPD markers.

Cluster analysis based on similarity matrices using UPGMA from RAPD data distinguished two groups, Kenana and Butana the first encompassed and second included Western Baggara and Nilotic with a genetic relationship of 80%. The genetic relationship within

each of the two groups were 82% and 85%, respectively. Within the local breeds the dendrogram distinguished two main groups within each of Western Baggara, Nilotic and Butana breeds. It also distinguished three groups within Kenana cattle, with a degree of genetic relationship ranging from 86% to 95% between the various strains within breeds.

The AFLP dendrogram constructed from UPGMA cluster analysis resolved three main clusters with a genetic relationship of 53%, 57% and 66% between Western Baggara and Nilotic; between Western Baggara and Butana; and between Butana and Kenana cattle, respectively. Within the 4 local breeds the genetic relationships were 61%, 67% 80% and 72% for Nilotic, Western Baggara, Butana and Kenana cattle respectively.

بسم الله الرحمن الرحيم

خلاصة الأطروحة

تم إستخدام نوعين من الواسمات الجزيئية RAPD و AFLP لدراسة التباينات الوراثية وتحديد البصمة الوراثية لعدد أربعة سلالات من الماشية المحلية السودانية هي أبقار غرب البقارة ، الأبقار النيلية، أبقار البطانة وأبقار الكنانة.

بإستخدام تقنيات الـ RAPD و AFLP ظهر تباين وراثي واضح داخل هذه السلالات مقداره 51.5% و 91.5% على التوالي، بينما أظهرت تقنية الـ RAPDتبايناً مقداره 22.9%، 36.8%، 24.4% و 41.4% داخل كل من السلالات الأربعة على التوالي.

فقط ثمانية من 22 بادئة (36%) أستخدمت في تقنية RAPD وعدد 11 توليفة من البادئات أستخدمت في تقنية AFLP تمكنت من تحديد واسمات فريدة لكل سلالة.

وقد أستخدمت المعلومات الناتجة من تقنيات الـ RAPD و AFLP لتقدير درجة التشابه الوراثي بإستخدام معامل جاكارد والتي تراوحت بين 78.4% إلى 84.8% بمعدل 81.6% ومن 46.9% إلى 80.3% بمعدل 63.6% على التوالي، بينما تراوحت من 92.9% - 98.9% بمعدل 95.9%، من 88.2% إلى 96.3% بمعدل 92.2%، من 88% إلى 98.7% بمعدل 93.3% ومن 80.3% إلى 97.2% بمعدل 88.7% داخل السلالات المحلية الأربعة على التوالي بإستخدام تقنية الـ RAPD.

أستخدم التحليل الإحصائي الهرمي مبنياً على التشابه الوراثي ومستخدماً UPGMA من المعلومات الخاصة بتقنية الـ RAPDفي رسم شجرة نسب توضح العلاقة الوراثية بين السلالات الأربعة وداخلها. وقد أظهرت شجرة النسب مجموعتين كنانة وبطانة في المجموعة الأولي وأبقار غرب البقارة والأبقار النيلية في المجموعة الثانية، وقد كانت العلاقة الوراثية 80% بين المجموعتين، بينما كانت 82% و 85% داخل المجموعتين على التوالي. وقد ميزت شجرة النسب أيضاً داخل السلالات المحلية مجموعتين رئيسيتين داخل كل من أبقار غرب البقارة، الأبقار النيلية وأبقار البطانة بينما ميز ثلاثة مجموعات رئيسية داخل أبقار

الكنانة وقد كانت درجة العلاقة الوراثية تتراوح بين 86% إلى 95% بين المجموعات المختلفة داخل السلالات المحلية.

بإستخدام تقنية الـ AFLP ميزت شجرة النسب ثلاثة مجموعات أساسية ذات درجة علاقة وراثية مقدارها 53%، 57%، 66% بين أبقار غرب البقارة والأبقار النيلية، وبين أبقار غرب البقارة وأبقار البطانة وبين أبقار البطانة والكنانة على التوالي.

في داخل السلالات المختلفة فقد كانت درجة العلاقة الوراثية 61%، 67%، 80%، 72% للأبقار النيلية، أبقار غرب البقارة، أبقار البطانة وأبقار الكنانة على التوالي.

LIST OF CONTENTS

	Page
Dedication	i
Acknowledgement	ii
Abstract	iii
Arabic Abstract	v
List of Contents	viii
List of Tables	xii
List of Figures	xiv
List of Plates	xvi
CHAPTER ONE: INTRODUCTION	
INTRODUCTION	1
CHAPTER TWO: LITERATURE REVIEW	
2.1. Cattle population	8
2-1-1 Origin and classification of African breeds	8
2-1-2 Cattle breed in Sudan	9
2-1-2-1 Northern Sudan	9
2-1-2-2- Southern cattle (Nilotic)	19
2.2 DNA markers, fingerprinting and genetic variability	20
2-2-1 Random amplified polymorphic DNA (RAPD)	25
2-2-2 Amplified fragement length polymorphism (AFLP)	31
CHAPTER THREE: MATERIALS AND METHODS	38
3.1. Animal material	38
3.2. Samples preparation	38
3.3. Isolation of genomic DNA	41

3.3.1. Extraction and purification of genomic DNA..... 41
3.3.2. DNA electrophoresis..... 43
3.3.3 Photography..... 45
3.4. Quantitation of DNA..... 45
3.5 DNA finger printing..... 47
3.5.1 Random Amplified Polymorphic DNA (RAPD) 47
3-5-1-1 Primers used in RAPD analysis..... 47
3.5.1.2. Preparation of PCR reaction..... 48
3.5.1.3. Optimization of RAPD conditions 50
3.5.1.4. PCR program and temperature profile..... 51
3.5.1.5. Electrophoresis of PCR products..... 54
3.5.1.6. Visualization, scoring and photography..... 54
3.5.2. Amplified fragment length polymorphism (AFLP) 54
3.5.2.1. Procedure of AFLP 56
3.5.2.1.1. Restriction digestion of genomic DNA..... 56
3.5.2.1.2. Ligation of adapters..... 56
3.5.2.1.3. Amplification of restriction fragments..... 58
3.5.2.2. Optimization of AFLP conditions 59
3.5.2.3. Gel analysis of AFLP reaction products..... 60
3.5.2.3.1. Preparation of denaturing polyacrylamide gel..... 62
3.5.2.3.2. Gel pre-run..... 65
3.5.2.3.3. Sample preparation..... 65
3.5.2.3.4. Sample loading 66
3.5.2.3.5. Gel electrophoresis..... 66
3.5.2.3.6. Silver staining..... 66

3.5.2.3.7. Exposure of film..... 68

3.6. Data analysis..... 70

3.6.1. Scoring of the data..... 70

3.6.2. Statistical analysis 70

CHAPTER FOUR: RESULTS

4.1 Levels of polymorphism..... 72

4.1.1. RAPD analysis..... 72

4.1.1.1. Optimization of RAPD conditions..... 72

4.1.1.2. Level of polymorphism as revealed by RAPD markers..... 72

4.1.1.2.1. Between breeds..... 72

4.1.1.2.2. Within breeds..... 80

4.1.1.2.2.1. Level of polymorphism within Western Baggara cattle (Nialawy) as revealed by RAPDs markers..... 80

4.1.1.2.2.2. Level of polymorphism within Nilotic cattle (Majock) as revealed by RAPDs markers..... 85

4.1.1.2.2.3. Level of polymorphism within Butana cattle as revealed by RAPDs markers..... 89

4.1.1.2.2.4. Level of polymorphism within the Kenana cattle as revealed by RAPDs markers..... 93

4.1.2. AFLP analysis..... 97

4.1.2.1. Optimization of AFLP conditions..... 97

4.1.2.2. Level of polymorphism as revealed by AFLP markers..... 98

4.2. Unique markers and Fingerprints revealed by RAPD and AFLP..... 98

4.2.1. Unique markers revealed by RAPDs..... 103

4.2.2. Unique markers revealed by AFLPs..... 103

4.3. Genetic similarity (Genetic distance) ……..……..…… 107

4.3.1. Genetic similarity revealed by RAPDs……..……..…… 111

4.3.1.1.Genetic similarity between local breeds as revealed by RAPDs….. 111

4.3.1.2. Genetic similarity within local breeds as revealed by RAPDs…... 116

4.3.2. Genetic similarity as revealed by AFLPs……..……..…. 118

4.4. Genetic relationship between and within the local cattle breeds……. 118

4.4.1. Cluster analysis of RAPD data……..……..…… 119

4.4.1.1 Genetic relationship between the local breeds…….....…. 119

4.4.1.2. Cluster analysis of RAPD data within the local breeds…….....…. 119

4.4.2. Cluster analysis of AFLP…..……..……..……..……..……..…. 120

CHAPTER FIVE: DISCUSSION

DISCUSSION…..……..……..……..……..……..……..... 128

CONCLUSION AND RECOMMENDATIONS ……..……..……..……... 139

REFERENCES…..……..……..…….....……..……..……... 140

LIST OF TABLES

Title No.

Table 3.1. Locality of the breed understudy…….. 40

Table 3.2: Sequence of (10-mer) RAPD primers used to detect polymorphism within local breeds ……..... 49

Table 3.3 The components of each PCR reaction in RAPD analysis 52

Table 3.4 Components of restriction Digestion of genomic DNA….. 57

Table 3.5. List of AFLP primers used in this study and their adapter sequence…….. 61

Table 3.6. Component amount and final concentration of 6% acrylamide solution……..... 64

Table 3.7. Steps solutions and time of silver stain…….. 69

Table 4.1: Primers used for RAPD……..... 74

Table 4.2: Select Primers used for RAPD markers between local 76

breeds.....
Table 4.3: Number of bands generated and percentage polymorphism as revealed by RAPDs..... 77
Table 4.4: Sequence of (10-mer) RAPD primers used to detect polymorphism within local breeds..... 81
Table 4.5: Number of bands generated and percentage polymorphism within Western Baggara cattle (Nialawy) as revealed by RAPDs 82
Table 4.6 Number of bands generated and percentage polymorphism within Nilotic cattle (Majock) as revealed by RAPDs..... 86
Table 4.7 Number of bands generated and percentage polymorphism within Butana cattle breed as revealed by RAPDs markers..... 90

Table 4.8 Number of bands generated and percentage polymorphism within Kenana cattle breed as revealed by RAPDs markers..... 94
Table 4.9. Sequences of the primers used 99
Table 4.10 Number of amplicons generated and percentage poly-merphism among local breeds of cattle revealed by AFLPs markers.. 100
Table 4.11 Unique markers revealed by RAPDs..... 105
Table 4.12. Unique makers revealed by AFLP..... 108
Table 4.13. Genetic similarity matrix between Western baggara-Nilotic; Butana and Kenana cattle computed according to Jaccard's coefficient based on RAPD data..... 112
Table 4.14. Genetic similarity matrix within Western Baggara cattle computed according to Jaccards coefficient from RAPD data..... 113
Table 4.15. Genetic similarity matrix within Nilotic cattle computed according to Jaccards coefficient from RAPD data..... 114
Table 4.16. Genetic similarity matrix within Butana cattle, computed according to Jaccards coefficient from RAPD data..... 115
Table 4.17. Genetic similarity matrix within Kenana cattle, computed according to Jaccards coefficient from RAPD data..... 116
Table 4.18 Genetic similarity matrix computed according to Jaccard's coefficient based on AFLP data between four local breeds 117

LIST OF FIGURES

Title	No.
Fig. 3.1. A flow – chart showing the steps followed to identify four local breeds of Sudanese cattle.	39
Figure 3.2. Molecular weight and concentration of the different bands of the DNA marker (Lambda DNA Hind III digest and θX 174 Hae III digest)	51
Figure 3.3 Diagram of PCR – RAPD programme.....	53
Fig. 4.1 RAPD histogram showing the level of polymorphism as revealed by each RAPD.....	78
Fig. 4.2 RAPD polymorphisms identified between breeds.....	79
Fig. 4.3 RAPD histogram showing the level of polymorphism within Western Baggara- Nialawy cattle as revealed by each RAPD primer	83
Fig. 4.4. RAPD polymorphisms identified within Western Baggara – Nialawy cattle.....	84
Fig. 4.5 RAPD histogram showing the level of polymorphism within Nilotic- Majock cattle as revealed by each RAPD primer	87
Fig. 4.6 RAPD profile within Nilotic – Majock cattle.....	88
Fig. 4.7 RAPD histogram showing the level of polymorphism within Butana cattle as revealed by each RAPD primer.....	91
Fig. 4.8 RAPD profile within Butana cattle.....	92
Fig. 4.9 RAPD histogram showing the level of polymorphism within Kenana cattle as revealed by each RAPD primer.....	95
Fig. 4.10 RAPD profile within Kenana cattle.....	96
Fig. 4.11 AFLP histogram showing the level of polymorphism as revealed by each AFLP primer combination.....	101
Fig. 4.12 AFLP profiles of four local breeds……..	102
Fig. 4.13 RAPD profiles showing examples of Unique Positive Marker and Unique Negative Markers.....	106
Fig. 4.14 AFLP profiles showing examples of Unique Positive Marker and Unique Negative Marker with primer combination.....	110
Fig. 4.15. Dendrogram showing genetic relationship between local	122

breeds (Kenana, Butana, Nilotic and Western Baggara –based on RAPD data constructed by UPGMA).....
Fig. 4.16. Dendrogram showing genetic relationship obtained from RAPD data constructed by UPGMA among Western Baggara cattle 123
Fig. 4.17. Dendrogram of genetic relationship obtained from RAPD data constructed by UPGMA among Nilotic cattle..... 124
Fig. 4.18. Dendrogram showing genetic relationship among Butana cattle breed based on RAPD data constructed by UPGMA..... 125
Fig. 4.19. Dendrogram showing genetic relationship among Kenana cattle breed based on RAPD data constructed by UPGMA..... 126
Fig. 4.20. Dendrogram of genetic relationship generated from AFLP data constructed by UPGMA among 9 lines of 4 local breeds 127

LIST OF PLATES

Plate	Title	No.
1.	Kenana cow.....	12
2.	Kenana bull.....	12
3.	Butana cows.....	15
4.	Butana bull.....	16
5.	Western Baggara (Nialawy) cow.....	18
6.	Western Baggara (Nialawy) bull.....	18
7.	Nilotic cattle (Majock)	21