June - 2011

JUNAGADH - 362 001
JUNAGADH AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE
DEPARTMENT OF AGRICULTURAL BOTANY

B.Sc. (Agri.)

MANJULA VERMA

BY

THROUGH BIOCHEMICAL AND MOLECULAR MARKERS

GENOTYPES OF BRINJAL (Solanum melongena L.)

MOLECULAR CHARACTERIZATION OF DIFFERENT
JUNAGADH - 362 001
JUNAGADH AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE
DEPARTMENT OF AGRICULTURAL BOTANY

B. SC. (AGRI.)
Manjula Verma

BY

THROUGH BIOCHEMICAL AND MOLECULAR MARKERS
"GENOTYPES OF BRIJNIAL (Solanum melongena L.)"
"MOLECULAR CHARACTERIZATION OF DIFFERENT"
June 2011

Junnagadh - 362 001
Junnagadh Agricultural University
College of Agriculture
Department of Agricultural Botany

B. Sc. (Agr.)
Manjula Verma

BY

Plant Breeding and Genetics

IN

(Agriculture)
Master of Science

OF

FOR THE AWARD OF THE DEGREE
IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
JUNNAGADH AGRICULTURAL UNIVERSITY

Thesis Submitted To The

"Molecular Characterization of Different Genotypes of Brinjal (Solanum melongena L.) Through Biochemical and Molecular Markers"
DEDICATED
TO
MY BELOVED FAMILY

MANTULA...
bands at 7 and 14 days after germination. The protein profile generated the maximum number of 8 bands. The protein analysis was done from the seedlings of 7 and 14 days.

Protein analysis was done through RAPD, ISSR and SSR primers. Analysis through RAPD, ISSR and SSR primers. Polyphenol oxidase, acid phosphatase, and (ii) Molecular markers.

Native-PAGE and four isoforms viz., esterase, peroxidase and Biochemical markers analysis through protein profiling using and molecular markers with two main objectives: (i) biochemical genotypes of brinjal (Solanum melongena L.) through biochemical and molecular markers. "Molecular characterization of different genotypes of brinjal (Solanum melongena L.) through biochemical was conducted to study "Molecular characterization of different belonging to the family Solanaceae with 2n = 24. An experiment vegetative crop of tropical and temperate parts of the world, brinjal (Solanum melongena L.) is the most important

ABSTRACT

JUNAGadh
JUNAGadh AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE
DEPARTMENT OF AGRI. BOTANY

DR. V.P. Chhotaria
Major Guide

Ms. Manjula Verma
Name of the Student

THROUGH BIOCHEMICAL AND MOLECULAR MARKERS
GENOTYPES OF BRINJAL (Solanum melongena L.)
MOLECULAR CHARACTERIZATION OF DIFFERENT
Genotypes were having purple colour and oblong shape. Genotypes, JBCR-08-10-1 and JBC-08-08, both these two genotypes, JBCR-08-10-1 and JBC-08-08, were observed between SSVT line and SSVT Green (72%) was observed between SSVT line and SSVT Green. The cluster analysis revealed two main clusters with highest similarity percentage similarity varied from 0.70 to 0.72. The cluster analysis for each primer and was ranged from 0.23 to 0.84. Genetic distance varied with an average 6.8 bands per primer. The PIC was calculated with all showed 100% polymorphism except one primer primers and all showed 100% polymorphism except one primer. A total of 68 bands were obtained from the ten ISSR cluster.1.

In main clusters, cluster I and II, with genotype PLR-1 similarity in

physiologic tribe consists of UPGMA method generated two

and OCP-09) demonstrated 100% polymorphic. Five primers (OCP-01, OCP-11, OCP-09, OCP-12) nonpolymorphic. Five primers while remaining three bands were

out of which 47 bands were polymorphic with an average of 7.4.

Seven RAPD primers amplified a total of 50 bands/alleles.

13-2 were found to be most diverse one.

were found to be 100% similar, while genotype PLR-1 and JBCR-

coefficient ranged from 0.40 to 0.93. JBC-08-08 and JBCR-176

with the similarity range of 0.46 to 1.00. Jackard's similarity

Pooled analyses of isoenzymes revealed two main clusters

Phosphatase were visualized on Native-PAGE at 12 DAG.

for peroxidase, four for polyphenol oxidase and five for acid

for esterase. The maximum numbers of four bands for esterase, three

DAG. The maximum number of four bands for esterase, three

bisphenol isoenzymes. The isoenzymes analysis was carried out at 12

Isoenzymes patterns were used for the characterization of
Improved crop productivity with wider and diverse genetic backgrounds to obtain
Ecomonic mapping studies and for the development of biennial
Genotypes. The information gathered would be helpful in
Genotypes. The information gathered would be helpful in
markers, and can be used for genetic diversity analysis of biennial
that molecular techniques are more accurate than biochemical
diversity and genotyping of 16 biennial Genotypes. The data revealed
molecular and biochemical markers resulted in developing highly
biochemical markers. The Genotype identification through
DNA fingerprinting techniques are more precise than

Thus, DNA fingerprinting techniques were found to be 100% similar.
JBR-02-11 and JBR-06-08 were found to be 100% similar.
found to be diverse from the other Genotypes. The Genotypes
dendrogram depicted, two Genotypes PLR-1 and GP-149 were
dendrogram clusters. From the remaining fifteen Genotypes in different sub-clusters. From the
consisted of only PLR-1 genotype, while Cluster II consisted of
revealed a dendrogram consisted of two clusters. First cluster
The pooled study of all molecular and biochemical markers

and 0.385 to 0.857 in SSR.
was ranked from 0.188 to 0.909 in RAPD, 0.170 to 0.724 in ISSR
more effective than RAPD. The similarity of 16 biennial Genotypes
among the studied technique ISSR and SSR seems to be

two main clusters.

on SSR data precisely organized sixteen biennial Genotypes into
primer was ranged from 0.000 to 0.778. The dendrogram based
polymorphism and 3.2 fragments per primer, PIC value for each
which 27 were polymorphic with an average of 86.0%
All the SSR primers amplified a total of 32 bands out of
Major Guide

(Prof. P. Chovatra)

Date: 09/06/2011

Place: Junagadh

This is to certify that the thesis entitled, "Molecular Characterization of Different Genotypes of Brinjal (Solanum melongena L.) through Biochemical and Molecular Markers" submitted by Manjula Verma in partial fulfillment of the requirements for the award of the degree of Master of Science (Agriculture) in Plant Breeding and Genetics of the Junagadh Agricultural University is a record of research work carried out by him under my guidance and supervision and the thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

CERTIFICATE

Junagadh
Junagadh Agricultural University
College of Agriculture
Main Oilseeds Research Station
Research Scientist (Castor)
(Dr. V. P. Chovatra)
Junagadh
Junagadh Agricultural University
College of Agriculture
Main Oilseeds Research Station
Research Scientist (Castor)
(Y. R. Chavda)

Date: 12/07/2011
Place: Junagadh

Studies
as required under the regulation for Post Graduate examination of Major and Supporting subjects held on successfully completed the comprehensive/Preliminary

This is to certify that MANJULA VERMA has

CERTIFICATE

JUNAGADH
JUNAGADH AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE
J.A.U., Junagadh
College of Agriculture
Department of Agri., Botany
Professor and Head
(C. K. Mandaviya)

J.A.U., Junagadh
College of Agriculture
Principal and Dean
(A. V. Bhat)

Junagadh
Junagadh Agricultural University
College of Agriculture
Main Oilseeds Research Station
Research Scientist (Castor)
(A. P. Chovate)

Date: 12/07/2011
Place: Junagadh

acknowledged.
received during the course of investigation have been fully
been submitted for any other degree. The assistance and help
and guidance and supervision and that no part of this thesis has
research work carried out by

MANJULA VERMA
PLANT BREEDING AND GENETICS

Submitted for the degree of M. Sc. (Agrt.) in the subject of
THROUGH BIOCHEMICAL AND MOLECULAR MARKERS
GEOTYPES OF BRINJAL (Solanum melongena L.)
MOLeCULAR CHArACTERIZATION OF DIFFERENT

This is to certify that the thesis entitled

CERTIFICATE – II

JUNAGADH
JUNAGADH AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE
JNÍ, Junagadh
College of Agriculture
Department of Agricultural Botany
Professor and Head

C. R. Mangalvada

JNÍ, Junagadh
College of Agriculture
Department of Agricultural Botany
Associate Professor & Head

C. R. Mangalvada

JNÍ, Junagadh
College of Agriculture
Department of Agricultural Botany
External Examiner

M. A. Vaddor (Co-Examiner)

JNÍ, Junagadh
Main Oilseeds Research Station
Scientist (Cason)

V. P. Chowta (Co-Examiner)

Date: 12/07/2011
Place: Junagadh

Approved.

...satisfactorily, we therefore, recommend that the thesis be approved. The performance of the candidate in the oral examination was satisfactory. The following members of the examination committee, the external examiner was deemed by the candidate before the external examiner was deemed by the examination committee. The following members of the examination committee, the external examiner was deemed by the candidate before the external examiner was deemed by the examination committee. The following members of the examination committee, the external examiner was deemed by the candidate before the external examiner was deemed by the examination committee.

This is to certify that the thesis entitled

CERTIFICATE - III

JUNAGADH
JUNAGADH AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE

Molecular Characterization of Different Genotypes of Brinjal (Solanum melongena L.) through Biochemical and Molecular Markers

Submitted by MANJULA VERMA
J.A. U. Junagadh
College of Agriculture
Main Office, Research Station
Scientist (Castor)
Major Guide and Research
(A. P. Chovita)

16/07/2011.

This thesis duly bound and corrected have been submitted on
the oral examination held on 12/07/2011. The final copies of
suggested by external examiner and the advisory committee in
as
"THROUGH BIOCHEMICAL AND MOLECULAR MARKERS"
"GENOTYPES OF BRINJAL (Solanum melongena L."
"MOLECULAR CHARACTERIZATION OF DIFFERENT"
Junagadh has made all corrections in the thesis entitled
Department of Agril. Botany, College of Agriculture, Junagadh,
M.Sc. (Agriculture), in Plant Breeding and Genetics,
This is to certify that MANJULIA VERMA, student of
Date: 16/07/2011

CERTIFICATE

JUNAGADH
JUNAGADH AGRICULTURAL UNIVERSITY
COLLEGE OF AGRICULTURE
I extend my heartfelt thanks to member of my advisory committee, Dr. A. Qamar, professor, Department of Agricultural Statistics, Jauagadh.

I express my sincere gratitude to Dr. C. L. Gandha, Principal and Dean, University, from the Chancellor and Director of Research and Dean, Faculty of Agriculture. Studies and Research, University of Agriculture, University of Agriculture.

I owe my greatest thanks to authorities of the Jauagadh Agricultural Research Station, Jauagadh, who, without his kind support and encouragement, has been possible to conclude this work. I deeply acknowledge the support and encouragement of Mr. M. Qureshi, Assistant Professor, Department of Agricultural Statistics, Jauagadh.

This manuscript is the result of proper guidance, supervision, criticism, encouragement and suggestions from the Advisory Committee and my advisor, Dr. A. Qamar, chairman of the Department of Agricultural Statistics (Caslon), Main Campus.

I have deep regard for member of my advisory committee, Dr. Q. A. Mehera, OIC, A. Qamar, Dr. A. Chaudhry, Dr. Aslam, Dr. A. Qamar, Dr. Aslam, Dr. Aslam.

I wish to express my heartfelt gratitude to all those who have helped me to complete this project, especially my advisor, Dr. A. Qamar, who has provided constant guidance and encouragement. The final product is a result of their valuable suggestions and encouragement.

I would also like to thank my family and friends for their support and encouragement throughout this project.

"All things are difficult before they are easy" only our keen determination makes them possible.
It is my great pleasure to thank Dr. H. L. Dhadi and Assistant Research Scientist, and Prof. (Miss) Lata Raval (Assistant Professor) College of Agriculture Junagadh, for their thoughts provoking words and guidance in my life. It is again my great pleasure to thank Dr. N. B. Patel, Dr. K. K. Sharma, Dr. G. V. Kulkarni and all the staff of department of Agriculture, Junagadh for their cooperation during the research work.

I have no words to express my sincere thanks to the Dr. L. K. Dhadi and Head of the Vegetable Research Station and Dr. I. J. Golani (Assistant Research Scientist) of the Vegetable Research Station, for providing brinjal genotypes to conduct the research work.

The moral support of my friend Mr. Omi Verma is also appreciated. I take this opportunity to thank him too, for providing a very friendly and supportive ambience in my life.

I am grateful to my senior friends, Madhulika, Deepak, Tengale, Ashwin, Thanvi, for their helpful suggestions during my stay at IAU. I owe my loving thanks to my nearest friend Anjali, Matlida, Farlin, Shamilla, Dhari, Jafpa and Shilker for their help and encouragement. I am especially indebted to my fellow classmates and juniors of College of Agriculture, Junagadh for their help and support.

I cannot find words to express my heartfelt gratitude to my beloved parents, Mr. B. L. Verma and Smt. Bhawanshawari Verma, my sweet sister Meenakshi Verma and my loving brother Vivek Verma whose constant affection and blessings made my path easier. Above all my humble and whole hearted prostration before God for his unprecedented favor upon me.

Place: Junagadh
Date: 02/06/2011

Manjula Verma
<table>
<thead>
<tr>
<th>CONTENTS</th>
<th>NO.</th>
<th>CAPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-6</td>
<td>1</td>
<td>INTRODUCTION</td>
</tr>
<tr>
<td>7-32</td>
<td>II</td>
<td>REVIEW OF LITERATURE</td>
</tr>
<tr>
<td>64-133</td>
<td>III</td>
<td>MATERIALS AND METHODS</td>
</tr>
<tr>
<td>134-138</td>
<td>IV</td>
<td>SUMMARY AND CONCLUSIONS</td>
</tr>
<tr>
<td>I-XVII</td>
<td>V</td>
<td>REFERENCES</td>
</tr>
<tr>
<td>I-A</td>
<td>A</td>
<td>APPENDICES</td>
</tr>
<tr>
<td>Term</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>AFLP</td>
<td>Amplified Fragment Length Polymorphism</td>
<td></td>
</tr>
<tr>
<td>ACP</td>
<td>Acid Phosphatase</td>
<td></td>
</tr>
<tr>
<td>ADH</td>
<td>Alkaline Dehydrogenase</td>
<td></td>
</tr>
<tr>
<td>ALP</td>
<td>Alkaline Phosphatase</td>
<td></td>
</tr>
<tr>
<td>Amp</td>
<td>Amino Peptidase</td>
<td></td>
</tr>
<tr>
<td>CAP</td>
<td>Ammonium Per Sulphate</td>
<td></td>
</tr>
<tr>
<td>AT</td>
<td>Adenine-Thymine</td>
<td></td>
</tr>
<tr>
<td>bp</td>
<td>Base Pairs</td>
<td></td>
</tr>
<tr>
<td>BBP</td>
<td>Bromophenol Blue</td>
<td></td>
</tr>
<tr>
<td>CAT</td>
<td>Catalase</td>
<td></td>
</tr>
<tr>
<td>CAPs</td>
<td>Cleavage Amplified Polymorphic sites</td>
<td></td>
</tr>
<tr>
<td>cDNA</td>
<td>Complementary DNA</td>
<td></td>
</tr>
<tr>
<td>COPH</td>
<td>Cophenic Values</td>
<td></td>
</tr>
<tr>
<td>CTAB</td>
<td>Distinctness, Uniformity and Stability</td>
<td></td>
</tr>
<tr>
<td>DAG</td>
<td>Days After Germination</td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
<td></td>
</tr>
<tr>
<td>dNTPs</td>
<td>Deoxynucleotide Triphosphate</td>
<td></td>
</tr>
<tr>
<td>DUS</td>
<td>Ethylene Diamine tetra Acetic acid</td>
<td></td>
</tr>
<tr>
<td>EDTA</td>
<td>Esterase</td>
<td></td>
</tr>
<tr>
<td>EST</td>
<td>Expressed Sequence Tag</td>
<td></td>
</tr>
<tr>
<td>Et al.</td>
<td>Ethidium Bromide</td>
<td></td>
</tr>
<tr>
<td>GC</td>
<td>Glutamate Dehydrogenase</td>
<td></td>
</tr>
<tr>
<td>GDH</td>
<td>Glutamate Oxaloacetat Transaminase</td>
<td></td>
</tr>
<tr>
<td>GOT</td>
<td>Hectare</td>
<td></td>
</tr>
<tr>
<td>ha</td>
<td>Hydrochloric Acid</td>
<td></td>
</tr>
<tr>
<td>HCl</td>
<td>ISSR Primer Index</td>
<td></td>
</tr>
<tr>
<td>IPI</td>
<td>Inter Simple Sequence Repeat</td>
<td></td>
</tr>
<tr>
<td>ISSR</td>
<td>Kilo-Bases</td>
<td></td>
</tr>
<tr>
<td>Term</td>
<td>Abbreviation</td>
<td></td>
</tr>
<tr>
<td>------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>Molar</td>
<td>Ml</td>
<td></td>
</tr>
<tr>
<td>Micrometer</td>
<td>μm</td>
<td></td>
</tr>
<tr>
<td>Weight Per Volume</td>
<td>W/V</td>
<td></td>
</tr>
<tr>
<td>Volume Per Volume</td>
<td>V/V</td>
<td></td>
</tr>
<tr>
<td>Arithmetic Averages</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unweighted Pair-Group Method With</td>
<td>UPGMA</td>
<td></td>
</tr>
<tr>
<td>Ultra Violet</td>
<td>UV</td>
<td></td>
</tr>
<tr>
<td>Melting Temperature</td>
<td>Tm</td>
<td></td>
</tr>
<tr>
<td>Tetra Ethyl Ethylene Diamine</td>
<td>T.E.E.D.</td>
<td></td>
</tr>
<tr>
<td>Tris-EDTA</td>
<td>TE</td>
<td></td>
</tr>
<tr>
<td>Tris-borate EDTA</td>
<td>TBE</td>
<td></td>
</tr>
<tr>
<td>Thermus Aquaticus</td>
<td>Tag</td>
<td></td>
</tr>
<tr>
<td>Tris-acetate EDTA</td>
<td>TAE</td>
<td></td>
</tr>
<tr>
<td>Tones</td>
<td>T</td>
<td></td>
</tr>
<tr>
<td>Sequence Tagged Microsatellite Site</td>
<td>STMS</td>
<td></td>
</tr>
<tr>
<td>Simple Sequences Repeat</td>
<td>SSR</td>
<td></td>
</tr>
<tr>
<td>SSR Primer Index</td>
<td>SPI</td>
<td></td>
</tr>
<tr>
<td>Single Nucleotide Polymorphism</td>
<td>SNP</td>
<td></td>
</tr>
<tr>
<td>Similarity for Qualitative Data</td>
<td>SIMQUAL</td>
<td></td>
</tr>
<tr>
<td>Similarity Index</td>
<td>SI</td>
<td></td>
</tr>
<tr>
<td>Sodium Dodecyl Sulfate</td>
<td>SDS</td>
<td></td>
</tr>
<tr>
<td>Sequence Characterized Amplified Region</td>
<td>SCAR</td>
<td></td>
</tr>
<tr>
<td>Nested Clustering Method</td>
<td>SAHN</td>
<td></td>
</tr>
<tr>
<td>Sequential, Agglomerative, Hierarchical, and</td>
<td>rpm</td>
<td></td>
</tr>
<tr>
<td>Resolution Per Minute</td>
<td>RFI</td>
<td></td>
</tr>
<tr>
<td>RAPD Primer Index</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Table No.</td>
<td>Title</td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>3.1</td>
<td>List of brinjal genotypes used in present study</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>List of chemicals used for molecular and biochemical study</td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>List of chemicals for Polyacrylamide Gel Electrophoresis</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>Reagents/chemicals used in present study for DNA isolation.</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>List of RAPD primers used in the present study</td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>List of ISSR primers used in the present study</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>List of SSR primers used in present study</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td>Rm values of banding pattern of protein profiling from 7 DAG brinjal seedlings.</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Rm values of banding pattern of protein profiling from 14 DAG brinjal seedlings.</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>Jaccard's similarity coefficient of 16 brinjal genotypes based on protein profiling.</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>Rm values of banding pattern of polyphenol oxidase and acid phosphatase isoenzyme from brinjal seedlings at 12 days after germination.</td>
<td></td>
</tr>
</tbody>
</table>
| 4.4      | Jaccard's similarity coefficient of 16 brinjal genotypes based on esterase, peroxidase, polyphenol oxidase and acid phosphatase isoenzymes.
<table>
<thead>
<tr>
<th>Page</th>
<th>Molecular marker based on pooled data of 16 binuclear genotypes, Jaccard's similarity coefficient of 16 binuclear genotypes.</th>
</tr>
</thead>
<tbody>
<tr>
<td>122</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes based on pooled data of RAPD-SSR and ISSR.</td>
</tr>
<tr>
<td>120</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes based on SSR data.</td>
</tr>
<tr>
<td>117</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes based on pooled data of polymorphisms, PIC and PI obtained by SSR primers.</td>
</tr>
<tr>
<td>107</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes based on ISSR data.</td>
</tr>
<tr>
<td>104</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes based on pooled data of polymorphisms, PIC and PI obtained by ISSR.</td>
</tr>
<tr>
<td>95</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes based on RAPD data.</td>
</tr>
<tr>
<td>91</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes based on polymorphisms, PIC and PI obtained by RAPD primers.</td>
</tr>
<tr>
<td>86</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes based on protein profiling and four isoenzymes.</td>
</tr>
<tr>
<td>83</td>
<td>Jaccard's similarity coefficient of 16 binuclear genotypes.</td>
</tr>
<tr>
<td>No.</td>
<td>Title</td>
</tr>
<tr>
<td>-----</td>
<td>-------</td>
</tr>
<tr>
<td>70</td>
<td>Dendrogram depicting the genetic relationship and 14 DAG protein profile data among 16 binural genotypes based on 7 DAG.</td>
</tr>
<tr>
<td>81</td>
<td>Dendrogram depicting the genetic relationship phosphatase isocynzymes peroxidase, polyphenol oxidase and acid-among 16 binural genotypes based on esterase.</td>
</tr>
<tr>
<td>84</td>
<td>Dendrogram depicting the genetic relationship profiles and four isozymes-among 16 binural genotypes based on protein.</td>
</tr>
<tr>
<td>86</td>
<td>Electrophoretic pattern of genomic DNA of 16 binural genotypes on 0.8% agarose gel.</td>
</tr>
<tr>
<td>93</td>
<td>Bands amplified by the RAPD primers and properties of poly morphic and monomorphic.</td>
</tr>
<tr>
<td>96</td>
<td>Dendrogram depicting the genetic relationship bands amplified by the ISSR primers.</td>
</tr>
<tr>
<td>106</td>
<td>Properties of poly morphic and monomorphic genotypes from ISSR data of 16 binural.</td>
</tr>
<tr>
<td>110</td>
<td>Dendrogram depicting the genetic relationship among 16 binural genotypes based on the ISSR data.</td>
</tr>
<tr>
<td>111</td>
<td>Dendic values against Jaccard's similarity coefficients from ISSR data of 16 binural.</td>
</tr>
<tr>
<td>132</td>
<td>Dendrogram depicting the Genetic Relationship among 16 binary genotypes based on pooled data of all biochemical and molecular markers.</td>
</tr>
<tr>
<td>128</td>
<td>Coefficients from pooled data of RAPD, ISSR, and SSR.</td>
</tr>
<tr>
<td>127</td>
<td>Dendrogram depicting the Genetic Relationship among 16 binary genotypes based on pooled data of RAPD, ISSR, and SSR.</td>
</tr>
<tr>
<td>123</td>
<td>Coefficients from SSR data of 16 binary genotypes against Jacard’s similarity</td>
</tr>
<tr>
<td>121</td>
<td>Dendrogram depicting the Genetic Relationship among 16 binary genotypes based on the SSR</td>
</tr>
<tr>
<td>119</td>
<td>Properties of polymorphic and nonpolymorphic bands amplified by the SSR primers</td>
</tr>
<tr>
<td>Plate No.</td>
<td>Title</td>
</tr>
<tr>
<td>----------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.1</td>
<td>Photographic image of five cultivars of brinjal in present study.</td>
</tr>
<tr>
<td>3.1</td>
<td>Protein profile generated using polyacrylamide gel electrophoresis at 7 and 14 days after germination.</td>
</tr>
<tr>
<td>4.1</td>
<td>Polyacrylamide gel electrophoresis of esterase and peroxidase isoenzyme at 12 days after germination.</td>
</tr>
<tr>
<td>4.2</td>
<td>RAPD markers of brinjal genotypes generated by OPA-01 and OPW-11 and OPC-05.</td>
</tr>
<tr>
<td>4.3</td>
<td>RAPD markers of brinjal genotypes generated by OPB-01 and OPC-09.</td>
</tr>
<tr>
<td>4.4</td>
<td>ISSR markers of brinjal genotypes generated by UBC-80 and UBC-813.</td>
</tr>
<tr>
<td>4.5</td>
<td>ISSR markers of brinjal genotypes generated by UBC-814 and UBC-815.</td>
</tr>
<tr>
<td>4.6</td>
<td>ISSR markers of brinjal genotypes generated by UBC-824 and UBC-856.</td>
</tr>
<tr>
<td>4.7</td>
<td>SSR markers of brinjal genotypes generated by smSSR11 and smSSR12.</td>
</tr>
<tr>
<td>4.8</td>
<td>SSR markers of brinjal genotypes generated by smSSR14 and smSSR15.</td>
</tr>
<tr>
<td>4.9</td>
<td>SSR markers of brinjal genotypes generated by smSSR17 and EEMS15 and EEMS16.</td>
</tr>
</tbody>
</table>
Introduction

CHAPTER
few important cucurbits, tomatoes, brinjal, chillies, potato, cabbage, cauliflower, pea and maximum emphasis has been given on important vegetables like than 70 vegetable crops are grown in our country, but the more than 285 $\$/day/cap is not achieved, still the consumption of vegetables production in India is 2.6%, still the consumption of vegetables increased. The annual growth rate of vegetables in very fast. India is the second largest producer of vegetables in the world next to China. Thus, the demand for vegetables are increasing antioxidants, basic food (carbohydrate and protein) towards supply of balanced diet, nutrition security. Emphasis is now shifting from supply of multivitamins, vegetables and minerals play significant role in food and vitamins, vegetables which are the rich source of nutrition by different vegetables in our country is productivity of different vegetables in the world are average productivity of more than 200 improved varieties. However, development and development in the country that resulted in research and development in the country that resulted in concentrated efforts have been made in vegetable

**Earle 1991**

contains 1.100 Mbp of DNA (2.4 P/2C; A. Rahman and nuclear genome is slightly larger than that of tomato and Nuclear and temperate parts of the world. The C. pekinense or brinjal, is one of the most important vegetable crops of commonly known as C. pekinense 'abergina' (2n=24),

The brinjal (Solanum melongena L.) (2n=24)
Eggplant is a native of the Indian sub-continent, with introducted eggplant to the west during the 15th century. It has been cultivated in Asia for over 1500 years. Among centers of eggplant origin (Gleedie et al., 1986), Indomalaya (19791), Indochina, China and Japan are the secondary centers. Sanskrit literature (Kallio, 1993; Hinata, 1986 and Khan, 1986) also mentions the medicinal and economic value of eggplant. India is a probable center of origin (Gleedie et al., 1986).

An annual in general (Northman, 1986), eggplant is a biennial which is grown as a semi-annual. It grows heterelycyclic condition. The plant is a binomial which is grown as a biennial. Cross-pollination generally occurs due to Dasuny et al. (2011). Cross-pollination generally occurs due to the normal way of fertilization although cross-pollination is also possible by insect (Northman, 1986), Lawrence and Chavan (1999). Anthocyanin is Northman (1986) and Lawrence and Chavan (1999). Anthocyanin is also compact growth habit and large leaves and perfect flowers and an ideal growing temperature between 22-30°C, and has an erect dwarf. Dwarf plants are put under var description and long slender types are included under var. serpentinum and egg-shaped cultivars are grouped under var. esculentum, the round or varietes under the species Solanum melongena. The round or long, slender types are Solanum melongena. There are three main botanical intracultures L. 'S. vulcan Dunal, S. nigra L., and 'S. xanthocarpum' and among them are Solanum melongena. The most important belong to the non-tuber bearing species. The most important belongs is an important family which includes tomato, potato, tobacco and The Solanaceae family to which S. melongena belongs is an.
The major brinjal producing Cultivars has an area of 58545 ha with an annual production of 987749 MT (Anonymous, 2008). The brinjal is grown in area of about 5.13 lakh ha with production of 8.450 lakh MT in India (Anonymous, 2010). The brinjal is also grown in 90% of the world production area and 87% of the world Asia has the largest eggplant production which comprise about 38% of the world. Brinjal occupies third position among vegetable crops.

Plate 1.1 Photographic image of brinjal plant

Produce a bitter taste and off flavor. High content of glycoalkaloids (20 mg/100 g fresh weight) from 0.37 mg to 4.83 mg per 100 g fresh weight. Generally, the glycoalkaloidal content in the Indian commercial cultivars ranged which are of wide occurrence in plants of Solanaceae family. The bitterness in eggplant is due to the presence of glycoalkaloids numerous small, soft seeds, which are edible, but are bitter. The fruit is botanically classified as a berry, and contains

1999).

Conserved throughout the world (Sarkarababu et al., Eggplant Germplasm Resources and collections have been well documented, evaluated and (Hinita, 1986).
Thus, polymorphism detection with identification and marker-assisted selection is very limited (Piace et al. 1999; Nunome et al. 2001). Therefore, polymorphism in legumes for applications such as quantitative trait localisation in legumes and interspecific lines has been reported (Nunome et al. 2003). Polymorphism among cultivars and interspecific lines has been reported (Nunome et al. 2003). However, in polyploids, a low frequency of polymorphism among polyploid species has been reported (Li et al. 1999). Some related cultivars with the genotype but also affected by environment (Li et al. 1997 and Raven et al. 1999). Even morphological data can be gathered via molecular techniques and are more abundant compared to morphological data at the molecular level (Li et al. 1997 and Raven et al. 1999). There are several molecular techniques are used to detect diversity at the molecular level (SSR, ISSR, RAPD marker system were used). The aims of the studies reported in this thesis were to analyze genetic diversity of legumes and germplasm with different molecular tools. For the investigation of genetic variation between S. melongena cultivars and its germplasm the largest producer of brinjal followed by Maharashtra and Bihar, Haryana, Punjab, Madhya Pradesh, West Bengal is the Haryana, Punjab, Madhya Pradesh, West Bengal is the largest producer of brinjal followed by Maharashtra and Bihar, Haryana, Punjab, Madhya Pradesh, West Bengal is the largest producer of brinjal followed by Maharashtra and Bihar, Haryana, Punjab, Madhya Pradesh, West Bengal is the largest producer of brinjal followed by Maharashtra and Bihar. The genetic diversity of legumes and germplasm was analyzed using ISSR, AFLP, and RAPD markers. The results indicated that the genetic diversity among the cultivars was higher than that among the interspecific lines. The genetic diversity was also influenced by the environment. The results of the study indicate that the genetic diversity of legumes and germplasm is high and can be used for further studies. The study also highlighted the need for the conservation of legumes and germplasm for future generations. The study also highlighted the need for the conservation of legumes and germplasm for future generations. The study also highlighted the need for the conservation of legumes and germplasm for future generations.
The various biochemical methods like protein profiling, isoenzymes etc are useful for varietal identification but they are highly influenced by the environmental factors. Both methods of genes, however, such studies in E. globulus are in their infancy. Further, this may facilitate the resistance traits. The markers can function as probes for the disease resistant to bacterial wilt caused by R. solanacearum. Those are linked to important agronomic traits especially those of E. globulus. Their aim is to identify molecular markers in E. globulus. Recently, Khashayarifar et al., 2000; Kashyap, 2002). Recently, Khashayarifar et al., 1999; Mace et al., 1998; Mace et al., 1999; Khashyap and Rezam, 1999; Mace et al., 1998). These have been very effectively shown genetic diversity and relatedness among various E. globulus (Ischihi). RAPD, AFLP and ARF and AP studies have also been reported in weedy S. indica as well as in advanced cultivars of E. globulus. DNA diversity, greater DNA polymorphism has so far with nuclear DNA diversity. Limited work has been done so far (1996; Varsanyi et al, 2000). Limited work has been done so far and is needed to high throughput in most laboratories (Powell et al., 1996). Particular markers are useful because they are highly variable, easy to use and amenable to high throughput in most laboratories (Powell et al., 1996).
Physiological relationship among binodal genotypes.

4. To know the degree of genetic divergence and to study
using RAPD, ISSR and SSR markers.

3. To examine the genetic diversity among binodal genotypes
Polyacrylamide gel electrophoresis (PAGE).

2. To study protein profiling of different binodal genotypes by
Genotypes using Polyacrylamide gel electrophoresis (PAGE).

1. To study the isoenzymatic pattern of different binodal
was undertaken with following objectives.

Keeping all above facts in view, the present investigation

maturity.

relatively cheap, eliminate the need to grow plants up to
advantages of the species identification in that they are rapid,
indepedent of environmental condition, which offers significant
DNA based molecular markers such as RAPD, ISSR, and SSR
are influenced by tissue specificity and developmental stage. The
have some disadvantages e.g. their are also profoundly
Review of Literature

Chapter II
2.1 Biochemical Markers

2.2 Molecular Markers

Review of Literature

Chapter II
Populations of three species in Solanum luteus brevipes, S.
measured genetic variation within and divergence among 32
accessions (Spoonor et al., 1994) employed enzyme electrophoresis to

Differences in phosphatase and glutamine synthetase (shown in detectable
amounts by the accessions, whereas the other enzymes (acid
phosphatase at local Pgm-1 and Pgm-2 and Pgm-1 and
Adh-2, glucose-6-phosphate isomerase at locus Gpi-2 and
Exo-7, alcohol dehydrogenase at locus
relationships of Solanum melongena. However, these enzymes were found to be
specific among the seven cytokin genealogical accessions of S. melongena in
Ishihara et al., 1994 (investigated isozyme variation from a process of gene duplication.
meiotic metaphase I. It is postulated that patterns A and B showed associations of 4 or 6 chromosomes at
Colombian accessions used in hybridization and accessions with
Colombian accessions from the USA, Central America and Peru had pattern A. Other
accessions from Central America, including the
found that all those accessions exhibited three main bands
isoenzymes of 15 wild and cultivated Capsicum accessions and
electrophoresis and specific in situ assay showed two, after discontinuous polyacrylamide
synthetase isozyme except for Mandragora humilis, which
S. menthaceum, Hyoscyamus niger) tested showed one glutamine

Spoonor et al., 1994) employed enzyme electrophoresis to

Differences in phosphatase and glutamine synthetase (shown in detectable
amounts by the accessions, whereas the other enzymes (acid
phosphatase at local Pgm-1 and Pgm-2 and Pgm-1 and
Adh-2, glucose-6-phosphate isomerase at locus Gpi-2 and
Exo-7, alcohol dehydrogenase at locus
relationships of Solanum melongena. However, these enzymes were found to be
specific among the seven cytokin genealogical accessions of S. melongena in
Ishihara et al., 1994 (investigated isozyme variation from a process of gene duplication.
meiotic metaphase I. It is postulated that patterns A and B showed associations of 4 or 6 chromosomes at
Colombian accessions used in hybridization and accessions with
Colombian accessions from the USA, Central America and Peru had pattern A. Other
accessions from Central America, including the
found that all those accessions exhibited three main bands
isoenzymes of 15 wild and cultivated Capsicum accessions and
electrophoresis and specific in situ assay showed two, after discontinuous polyacrylamide
synthetase isozyme except for Mandragora humilis, which
S. menthaceum, Hyoscyamus niger) tested showed one glutamine

Spoonor et al., 1994) employed enzyme electrophoresis to

Differences in phosphatase and glutamine synthetase (shown in detectable
amounts by the accessions, whereas the other enzymes (acid
phosphatase at local Pgm-1 and Pgm-2 and Pgm-1 and
Adh-2, glucose-6-phosphate isomerase at locus Gpi-2 and
Exo-7, alcohol dehydrogenase at locus
relationships of Solanum melongena. However, these enzymes were found to be
specific among the seven cytokin genealogical accessions of S. melongena in
Ishihara et al., 1994 (investigated isozyme variation from a process of gene duplication.
meiotic metaphase I. It is postulated that patterns A and B showed associations of 4 or 6 chromosomes at
Colombian accessions used in hybridization and accessions with
Colombian accessions from the USA, Central America and Peru had pattern A. Other
accessions from Central America, including the
found that all those accessions exhibited three main bands
isoenzymes of 15 wild and cultivated Capsicum accessions and
electrophoresis and specific in situ assay showed two, after discontinuous polyacrylamide
synthetase isozyme except for Mandragora humilis, which
S. menthaceum, Hyoscyamus niger) tested showed one glutamine
different band patterns. Seven band patterns (B1, B2, B3, B4, B5, B6, B7) were resolved, giving 12
subterminal. A total of 19 major bands were resolved giving S. aethiopiacum, S. macrocarpon, S. melongena, S. nigrum, and S.
taxonomic relationships among the five species namely S.
variations based on total seed proteins and to infer the
the Philippines using SDS-PAGE to determine the genetic
of the different non-tuber forming Solanum species collected in
One et al., (1996) studied two hundred twenty accessions
monomorphic or in similar frequencies in insanum and insanum
monomorphic, and nearly all of the genes were either also
monomorphic, and nearly all of the genes were either also
for 29 isozyme loci. In S. melongena, 22 of the 29 loci were
accessions of wild forms (referred to as "insanum") were surveyed
accessions of wild forms (referred to as "insanum") and two
accessions/taxon. Twenty-nine accessions of S. melongena, 33
species/taxon. Twenty-nine accessions of S. melongena (EGPplant) and similar wild and
cultivated Solanum melongena(shown in
Karthikeyan et al., (1995) studied enzyme electrophorograms in
found in S. brevidens and S. euberosum at three other loci.
local, in addition, it possesses only a subset of the variability
S. euberosum results from novel alleles at two of the 12 isozyme
S. euberosum and S. brevidens (0.780) and S. euberosum
more divergent to both S. brevidens (0.968) and S. euberosum
Solanum fernandezianum showed no heterozygosity and was
species monomorphic for alleles at nine of the 12 loci examined.
interpopulational values (0.923, 0.855, respectively), with both
brevidens and S. euberosum (0.00-0.04) were similar to their
three species. Interpopulational mean 
 heterozygosity was
among all euberosum, and S. fernandezianum. Very low levels of observed
and S. fernandezianum.
transpherase (GOT), and malic enzyme (MDH), using a cluster
6-phosphogluconate dehydrogenase (6-PGDH), aspartate amino
enzymatic systems, including esterase (EST), peroxidase (PRX),
one accession of the genus Capsicum with five polymorphic
Barrera et al. (2005) evaluated two hundred and sixty-
phytochrome.
showed positive tests, indicating that catecholase is a pronounced
cathchol and prolino, basic huchinu and ethidium bromide
ehibited catecholase activity, when stained with 4-methyl
point of application and other near dye front. These bands
bands on staining with Coomassie Brilliant blue, one at the
Salmonella melongena (brinjal) on natural habitat, ligation, the elution
Cosimari et al. (2003) pulsed catecholase from cortex of
mucilaginous, and S. nigra
opened to S. aethiopicum followed by S. macrocarpon, S.
closely related to S. aethiopicum followed by S. macrocarpon, S.
through cluster analysis, S. melongena was found to be the most
similarity index values and a single linkage dendrogram obtained
wild Salmon melongena species, to S. melongena was inferred based on
four neighboring provinces. The taxonomic relationships of the four
sample size and the use of accessions originating from
observed among the wild species probably due to the small
and in S. nigra (BPI8, BPI11), while one each in S. macrocarpon
and in S. nigra (BPI8, BPI9, BPI11), while one each in S. aethiopicum
and two patterns each were observed in S. aethiopicum (BPI, BPI9)
Except for BPI, the band patterns were specific to S. melongena,
sufficient genetic diversity among the local eggplant collection.
BPI4, BPI5, BPI7, BPI9, BPI10, BPI11, BPI12, BPI13, BPI14, BPI16, BPI17, BPI18, BPI19, and BPI20 were exhibited by S. melongena indicating...
PAGE gave 30 protein bands in the 6.5-205 KDa molecular
profile. In this study, the source of plant materials was
varieties under study or their hybrids. Tubers of potatoes
certain bands could be easily used by researchers to identify
dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE).
patterns of 13 varieties of potato (Solanum tuberosum)
compared storage protein banding
Al et al. (2007) transaminase, transaminase.
the enzymes except malic enzyme and glutamate oxaloacetate
Culture specific isozymic banding patterns were observed for all
polymorphic with six and eight banding patterns, respectively.
acid-phosphatase and malate dehydrogenase were highly
banding patterns were observed for malic enzyme.
transaminase exhibited three banding patterns while two
enzymes, dehydrogenase, esterase and glutamate oxaloacetate
seven-day-old seedlings grown under greenhouse conditions.
used to analyze extracts prepared from young leaf tissues of
accessions and three cultivars. Starch gel electrophoresis was
Phyllostegia biflora by studying six enzymes among 16 binucle
Phyllostegia et al. (2005) assessed isozymic variability in
followed by PRX, 6-PGDH and GLO.
bands. The most polymorphic enzymes were EST and the ME,
bands. GOT with 8 bands; ME with 17 bands and EST with 37
whole set of samples as: PRX with 15 bands; 6-PGDH with 8
enzymatic systems showed a total of 83 bands, distributed in the
cumbersome and C. phlebas did not group independently, The five
banding patterns were observed, while the species C. annuam, C.
characterized. Grouping of the species C. bacaccum and C.
Suggested for G-6-PDH 2 and SKDH 1 loci. However, tetrasomic inheritance was
more frequent, their selection ratios did not fit in with any of the expected
isozyme markers segregated in the dihaploids in a distorted
inter simple sequence repeat (ISSR) markers were evaluated. The
inheritance and segregation ratios of three isozyme systems and
phenotypically analyzed. The exent of diosomic/tetrasomic
heterogeneity was evaluated. The exent of diosomic/tetrasomic
culture of the corresponding tetraploid somatic hybrids was
another marker. A dihaploid population obtained through another
Solanum aethiopicum by explanting biochmical and molecular
recombination between two species, Solanum melongena and
S. tuberosum et al., (2008) evaluated the extent of genetic
most dissimilar one was Roma
Romano and Konidor varieties were the most similar, but the
Cosmos, Herre, Roma, Konidor and Diota St, and Picasso;
Pima, Bolesta, Marion, Diamant and Bright (3). Agne,
0.06). Cluster analysis differentiated 13 varieties in four groups:
0.34 = between potato varieties is smaller than their similarity with a mean value of 0.34, showing that the genetic dissimilarity
classified together between around 0.16 to 0.52 level of distance,
estimate the genetic distance among the varieties. They were
protein present in a culture, protein profiles were used to
patatin appeared to be useful indicators of the presence of
KDa position was identified as patatin. Both sporanin and
40 or the storage proteins, sporanin. Another thick band (A)
at 23 to 25 KDa positions could be probably the isomers
prominent bands and 15 minor bands. Three major bands (B, C
weight range, including four major bands (A, B, C, D).
Dendrogram of *Capsicum* (12), occupies a distinct place as revealed in the
Cluster-I, comprising of two species each. *Capsicum annuum* and *Solanum lineare*. A dendrogram constructed based on the UPGMA
clustering method revealed two major clusters, Cluster I and
Cluster-II, each comprising of two species each. The species *Capsicum annuum* and *Solanum lineare* are more closely related to each other
than *Capsicum annuum* and *Solanum melongena* which are cultivated and wild,
respectively. The present protein profile revealed that the experimental genus *Lycopersicon* is very close to the genus
*Capsicum annuum* or family Solanaceae through polypeptide gel electrophoresis and using cytological characters viz. mitotic
prophase of *C. annuum* and *S. melongena* S' is more close at molecular level as compared to other species.

Bhat et al. (2011) investigated cytological characters and

respective.

Groups A, B, C, and D were clustered in three
100%. Nineteen *Capsicum* genotypes were clustered in three
alleles / loci. Estimates of genetic distances (GD) ranged from 0 to
12.5% using SDS-PAGE analysis.
1990's. Reduction in the genetic diversity among modern tomato
1970's exhibited significantly fewer levels or marker diversity (mean = 0.165)
and low levels of pairwise similarity (Jaccard's mean 0.825). The overall high levels of
the existence of limited genetic variation in the
cultivars using RAPD markers, generated by 42 random primers,
Arachch et al. (2002) analyzed genetic diversity of 27 tomato
maps have been established by using RAPD and RFLP markers.
Two genetic linkage studies based on AFLP and cDNA analyses, between
Collonier et al. (2001) analyzed the genetic relationships
to distinguish them taxonomically;
it was no longer appropriate
were highly diverse morphologically; it was also no longer appropriate
the results showed that even though S. melongena and Insanum
survey performed on the same accessions. The concordance of
do not have a high similarity between them was 0.947. The RAPD results were
more diverse than those of S. melongena. The calculated
found in S. melongena. Overall, the Insanum accessions were
insanum displayed an additional 13 fragments which were not
all of which were also present in Insanum. The
130 fragments of Solanum melongena exhibited 117 of the
weekly forms known as "Insanum", "Rapal", and "Rapid"
Karthikeyan et al. (1994) carried out RAPD analysis on fifty-

2.2.1 RAPD

2.2 MOLECULAR MARKERS
close relationships of *C. thaliana* (RAPD data). Molecular data also supported the
similarity of *C. thale* with *C. annuum* and *C. capsicum*. Molecular markers revealed high genetic
bootstrap values. Molecular markers were used to separate species with high
of *C. thale* and *C. capsicum* and the *C. annuum* species. The *C. capsicum* data confirmed the recognition
of the *C. capsicum* species. The genetic analysis of 61 accessions representing 11 species of
the *C. capsicum* species. In total, 1921 bands were used in
determine genetic variation and phylogenetic relationships
Kochi et al. (2004) used RAPD marker system to

in *S. torum*

further genetic and epigenetic studies on bacterial wilt resistance
from beyond as reference. Such results could be essential for
revealed by the genetic study of seven accessions of *S. torum*
using 168 RAPD markers. Only 1.2% polymorphism was
was confirmed by the genetic homogeneity (zero% polymorphism)
accessions regarding high bacterial wilt tolerance and its result
Chen et al. (2004) revealed homogeneity between

geographical distance.

Mantel test showed no correlation between genetic distance and
the variation within species and groups was more than 90%, A
variation among species and groups was less than 10%, whereas the
AMOVA analysis of the genetic variation showed that the
An *aethiopicum* group and *S. aethiopicum* showed that the
RAPD markers for 18 populations of *Solanum aethiopicum* S
Steele et al. (2003) studied variation in 39 polymorphic

for similar plant and fruit characteristics.
cultivars may be attributed to the recent trend towards breeding
Genetic structure of 15 wild populations and three domesticated

Oyama et al. (2006) studied levels of genetic variation and

Genes.

the hybrids and also to monitor introgression of the useful
hybridization. Molecular markers can be employed to identify

sympatric (Solanum insulileafnum, S. incanum, S. integrifolium and S.

Solanum melongena) and four related non-lupines species

of Solanum melongena and show the high genetic diversity from 96 accessions including 92

Bharya et al. (2006) used 23 STMS primers for the

assessments of genetic diversity. Between the cultivated varieties and the wild types.

exists between the cultivated varieties and the wild types.

obtained from the RAPD markers showed the genetic variation
obtained which exhibited 54.7% polymorphic bands. A total of 67 amplified products were

polymorphic bands. A total of 67 amplified products were

found to be highly informative and they produced distinct and

total of 31 arbitrary primers screened, only seven primers were

amplified. Amplified DNA (RAPD) markers were

random amplified polymorphic DNA (RAPD) markers. Among

two wild species Solanum torvum and Solanum violaceum using

among Mahattan elite plant (Solanum melongena) varieties and

Seraye, B. (2005) carried out survey of the genetic diversity

groups. The resulting dendrogram divided the accessions into two major

values among the studied genotypes range from 0.209 to 0.891.

amplified polymorphic DNA (RAPD) markers. The similarity

Sithirivong et al. (2005) screened ten pepper (Capsicum

member of the C. annuum complex. and this species could therefore be considered to be a

analyses) and this species could therefore be considered to be a
reactions. The OBP161193 marker was confirmed in the five independent PCR
OBP161193 marker were used in this study. Reproducibility of the
hybrids of tomato, viz., NTH-1 (DVRT-Fore Dade) and NTH-7
purity testing in tomato (Solanum lycopersicum). Two potential
Singh et al., (2007) studied RAPD markers for hybrid seed
improvement.
could be potential sources of germplasm for eggplant
categorially distinct genotypes identified using RAPD markers
and a dendrogram constructed by UPGMA method showed that
indicated presence of high level of genetic diversity in eggplants
coefficient ranged from 0.05 to 0.82. The similarity results
discriminated all the accessions. The value of Jacard’s
products were obtained from 14 deacem primer, which
representing five species. A total of 144 polymorphic amplified
and species relationships among 28 accessions of eggplant
dNA (RAPD) technique as a tool for assessing genetic diversity
Singh et al., (2006) used random amplified polymorphic
and 51.1% (population in both wild and domesticated samples.
equally distributed within (48.9 and 50.0%) and among (50.0%
populations, ANOVA indicated that total genetic diversity was
0.165 for wild populations and 0.081 and 0.131 for domesticated
populations. Mean and total genetic diversity were 0.069 and
34.2% in wild populations and 34.7% in domesticated populations of Capsicum annuum.
166 band (all of them polymorphic) and 126 bands (12.5% of them
polymorphic) were amplified in wild and domesticated
populations of C. annum by RAPD markers. A total of
same locus. BSA (BSA) was performed
interfertility indicated that these two genes were alleles of the
between the resistance genes from S. acetobacterium and S.
Toppino et al. (2008a) studied the allele relationship
polymorphisms in 81 out of 85 bands amplified.
the genotypes. However, nine RAPD primers showed
the genotypes. However, nine RAPD primers yielded invent.
profiles for all
species of the 55 RNAs yielded invariant banding profiles for all
individuals. To assess genetic variation, the non-transcribed
untranslated regions were sequenced by assessing Jordanian epgent
information and reference to phylogenetic studies.
information and reference to phylogenetic studies.

Polatco and Solanun. This investigation provided new
ancestrally, Minon (Syn. Breviarticum, leptospermum,
distances were estimated for 42 accessions from five subspe-
random amplified polymorphic DNA (RAPD) technique. Genetic
and genetic variation in the genus Solanum based on the
Poczael et al. (2008) examined phylogenetic relationships
the same side as the other two markers.
Gene. RAPD marker H12 mapped 2.6 CM from this gene and on
polymorphism. Three markers were linked within 2.6 CM of the
was screened with 2.316 primer combinations to detect
population of epgent selected for Fusarium wilt resistance
the monogenic inheritance of resistance. DNA from F2 and BC1
RGA and RAPD markers. Analysis of segregation data combined
resistance to Fusarium wilt (Fom) in epgent using SREP, SREP-
Multi et al. (2008) reported the genetic of the gene for
NTH-1.
NTH-1. NTH-1. NTH-1. NTH-1. NTH-1. NTH-1. NTH-1. NTH-1.
of 19 advanced cultures and landraces of brinjal using RAPD

Twari et al. (2009) performed molecular characterization

diversity among ten commercial varieties of chilli in India.

differential markers. This effort can serve determining

were polymorphic for a specific primer and can be used as

Around 200 different bands were observed under UV light. Seven

DNA solution was subjected to agarose gel electrophoresis.

successive PCR cycles. Using 12 different primers, the

amphiphilic
dNA (RAPD) analysis. The DNA extracted

ten different commercial varieties of chilli using random

makari et al. (2009) investigated the genetic diversity of

potential tool for epsplant improvement.

genetically distinct varieties using RAPD markers could be a

all loci were 57.89% and 0.23, respectively. Identification of

proportion of polymorphic loci and gene diversity values across

obtained of which 44 fragments considered polymorphic. The

selected. With these primers, 76 clear and bright fragments were

varieties of epsplant. Out of 21 primers scored four were

assessing genetic diversity and varietal relationships among ten

biswas et al. (2009) used RAPD technique as a tool for

for indirect selection of F1 hybrid resistance in epsplant.

in segregating progenies revealed that they represent useful tools

efficiency of these markers in predicting the resistant phenotype

the basis of the amplified sequences. The evaluation of the

polymorphic sequences (CAPPS) were subsequently obtained on

the resistance trait were identified. Cleaved amplified

backcross progenies, and three RAPD markers associated with

with RAPD markers on the BC3/BC5 resistant advanced
Genetic Relatedness of the Genotypes.

PCR and RAPD markers were used to examine the polymorphism of the UPGMA dendrograms. The rest of the primers gave less than 50% polymorphic bands, the rest of the genotypes were classified into two groups. The number of bands per primer ranged from seven (OPH19, OPH20, OPH30) to four (OPB07). The number of bands per primer ranged from 26 to 10, and the number of bands per primer ranged from 10 to 12. Primer OPB07 was the most polymorphic. Sixty-four percent of the primer bands were amplified, among which 29 were polymorphic. The number of bands per primer ranged from 7 to 4. Using 11 SSR and RAPD markers, U. susinii collected from different geographical locations were classified using RAPD and SSR markers with a molecular characterization.

Genetic Specificity and Diversity.

The basis of RAPD fingerprinting, which showed a level of specificity, was calculated as 8.94 x 10^-4. The probability of chance identity between two cultivars for RAPD markers was calculated as 0.873. The number of cultures distinguished by RAPD was r = 0.873. The correlation between RAPD and the number of amplified fragments of these 66 (27.5%) RAPD fragments were used within 100% of a total of 240 markers. Twenty-nine RAPD markers generated a total of 240
Lycopersicon cheesmanii, Lycopersicon humboldtii, Lycopersicon pennellii, and two Lycopersicon esculentum subsitution lines (Il

Tiwana et al., (2003) studied the level of polymorphism in
tomato using ISSR-PCR with 14 primers. Five tomato species

The analysis for genotyping and phylogenetic studies in tomato,

and molecular evidence, supporting the applicability of ISSR

consistent with Lycopersicon taxonomy based on morphological

ISSR patterns was low. The ISSR-based phylogeny was generally

obtained for L. esculentum, although the interspecific variation of

number (more than 20) of species-specific fragments was

fragments were detected for each tomato species. The highest

ISSR primers was 95.6%. Species-specific ISSR

wild and cultivated tomato genomes. The polymorphism revealed

nucleotides. In total, 318 ISSR fragments were amplified for the

microsatellite repeats and containing additional selective markers

Lycopersicon. Analyses involved 14 ISSR primers homologous to

relationships in 54 wild accesses and cultivars of the genus

ISSR) analyses to study the genetic diversity and phylogenetic

Kochiha et al., (2002) used inter-simple sequence repeat

distinguish all the four disturbed samples unanimously.

141 bands per primer. These four primers could reliably

using three of the 14 primer with each primer with an average of

The ISSR-PCR assay revealed a total number of 366 bands

unambiguously differentiate all the four disturbed chikil samples.

primers and four out of eight mononucleotide primers could

seven of the 14 primer with each primer with an average of

the analyses. A total of 212 and 288 bands were resolved by

...
melongena) and 12 accessions in eight related Solanum species
repeat (ISSR) analyses in eight cultures of eggplant (Solanum
Ishikawa et al. (2008) performed inter-simple sequence

An informal classification of Capsicum can be proposed.
Complex combining the results based on molecular data
obtained in this study and results obtained by other researchers
could therefore be considered to be a member of the C. annuum
C. galloagraceae with C. annuum (ISSR analyses) and this species
ISSR data), Molecular data supported the close relationships of
high genetic similarities of C. pubescens with C. pratttiissum
species with high bootstrap values. Molecular markers revealed
the recognition of C. pubescens and C. chinense as separate
dendrograms were congruent but not identical in the clustering
11 species of the Capsicum genus, AFLP, RAPD and ISSR
were used in the genome analysis of 61 accessions representing
relationships within the genus Capsicum. In total, 1921 bands
were used to determine genetic variation and phylogenetic
Kochi et al. (2004) used AFLP, RAPD and ISSR marker

Having some new bands compared with ISSR fingerprints,
RGA (Resistant-Gene analogs) primer resulted in fingerprints
Li copersicron esculetinum chlorosomes 6, Using one ISSR and one
enabled to place thirteen ISSR markers on the classical map of
Li copersicron esculetinum substitution lines 6-3 and WSL 6
previous phylogenetic investigations. ISSR-PCR on two
previous phylogenetic investigations. The data showed complete correspondence to
tomato species. The tree showed four main branches of the tree
primers were individually able to distinguish all tomato species.
6-3 and WSL 6 were analyzed. Nine out of these fourteen
specific primer. The isolates were classified into groups and their identity was confirmed by PCR amplification using the specific primer. Pathogens were identified by their morphological characteristics.

Baydak et al. (2010) used ISSR markers to characterize cultivars for ISSR markers was calculated as 2.25 x 10^-2. The probability of chance identity between two ISSR primers was 0.06. The probability of culturing distinguished by ISSR primers was and the number of cultivars distinguished by ISSR primers were polymorphisms. The correlation between primer RPs of the 299 fragments produced by ISSR markers. Twenty-three anchored and non anchored ISSR markers. Seventeen advanced cultivars and landraces of prunial using ISSR

The selection of ISSR primers was inherited according to random monomorphic ISSRs were inherited according to random the tetrasomic and/or disomic inheritance. Twenty-four of the 66 (simple/duplex/triplex), almost 64% of the fragments revealed 71 dihaploids. According to the genetic constitution of 104 melongena-specific, and 66 monomorphic (110 of the 70 melongena and its allied Relative Solanum aethiopicum, Solanum melongena and its allied Relative Solanum aethiopicum, the extent of genetic recombination between the two species the phylogenetic study and the cultivar identification.

The phylogenetic study and the cultivar identification. The ISSR analysis was demonstrated to be available for 99.1%. The ISSR analysis was performed to assess the phylogenetic relationships and identify the cultivars. A total of 52 polymorphic amplified bands were obtained from 34 of the 100 isolates tested, and the percentage of polymorphisms was 99.1%. The ISSR analysis was demonstrated to be available for 99.1%. The ISSR analysis was performed to assess the phylogenetic relationships and identify the cultivars. A total of 52 polymorphic amplified bands were obtained from 34 of the 100 isolates tested, and the percentage of polymorphisms was 99.1%.
2.4.2. SSR

2.2.3 SSR

...and the application for certification purposes. The importance of guaranteeing the differentiation of chili cultivars UBC-809 (34) and UBC-66 (53), this study revealed the Great percentage of polymorphic bands obtained by the use of primers reproducible bands of which 139 were polymorphic. The simple sequence repeat (ISSR) five ISSR primers amplified 204 screening DNA from thirteen Capsicum cultivars using inter-specific and diversity.

on the basis of ISSR fingerprints, which showed a level of genetic...
systems for studying genetic diversity in tomato and pepper. Among all results, SSRs are most informative of the three polymorphic systems (SSR) and simple sequence repeat (SSR) markers were compared to that of amplified fragment length polymorphism (AFLP) markers in the utility of SSR polymorphism (SSAP) marker system. The utility of SSR recombination sequence-based amplicon-specific sequence-accumulation collections of tomato and pepper industrial lines by Tan et al. (2005) assessed the genetic diversity of gene-trait relationships in tomato.

Identification and marker-assisted selection, but for identifying gene-trait relationships, they can be used not only for molecular mapping, culture developed in this study are primarily from expressed sequences. The set of 196 polymorphic SSR loci. Since the markers in tomato cultivars were clustered based on the banding patterns, only 6.2% had tetra- and pentanucleotide repeats. The set of 196 microsatellite loci contained di- or tri-nucleotide repeats and 33.8% had three alleles. The vast majority (93.8%) of the 2.7 alleles per locus; 49.2% of the local had two alleles, and six SSR alleles were detected for each locus with an average of 2.7 alleles per locus; ranging from 0.99 to 0.67. Among the polymorphic loci, two to four alleles were polymorphic with the polymorphism information content (PIC) of these loci were 0.029. PCR products and expected DNA fragments in their PCR products, and 65 of them were polymorphic with the expected DNA fragments. The 158 pairs of SSR primers selected against a set of 19 diverse tomato for SSRs and analyzed for the design of PCR primers. Of these SSRs, 190 pairs yielded PCR products for each locus. He et al. (2003) searched the hundred DNA sequences of S. melongena which is in agreement with its very close relationship with S. melongena.
Arithmetic Mean (Upcma) clustering at genetic similarity value content (PIC) was 0.31. Unweighted Pair Group Method with average linkage was used and the average polymorphism information content was 4.3. The mean number of alleles per locus was 150 alleles were found with moderate levels of diversity, and a high number of unique alleles polymorphic markers, a total of 120 alleles were found with polymorphic markers, a total of 110 alleles were found with SSR from China, Japan, South Korea, and USA. Using 35 SSR determinants and 35 SSR determinants of the genetic diversity of 39 Accessions a high similarity value was observed, diversity was found to be high. The results of AFLP studies showed that correlation between samples was significant, the integral value of similarity was found to be 0.88. This indicated the statistical results of SSR analysis; the "I" value of "I" value of polynomic data was found to be 0.49. This indicates the variation was observed between samples. According to the results in the best scale, these results showed that 64.34% of the wild relatives, SSR markers system was used. For the AFLP data investigation of genetic variation between Solanum melongena and its wild relatives, SSR marker system was applied to the same sample genotypes and for the marker system was applied to the same sample genotypes and for the Tumblin et al., (2007) analyzed genetic diversity of Tumblin et al., (2007) analyzed genetic diversity of wild relatives and wild relatives with different molecules with a significant correlation of genetic relationships between different molecular tools. To reveal genetic diversity among eggplant cultivars, AFLP tools reveal specific genetic relationships, while SSR has the ability to genetic variation and relationships, while SSR has more suitable for investigating overall markers. For tomato, SSR is more suitable for integrated overall with a significant correlation of genetic relationships between different SSR data sets and between SSR, AFLP and SSR.
that there was a high heterogeneity among the British varieties for the control cultivars it was 1.4. The results clearly confirmed the microsatellite loci. The average number of alleles for British varieties was 1.3, for the varieties from the CGN it was 0.4, and for the Chinese varieties was 0.3. The cultivars obtained from the CGN were more or less homogeneous with monomorphic loci, while the cultivars obtained from China were more diverse. The cultivars obtained from the Centre for Genetic Resources of the Netherlands (CGN), Tomato cultivars (TOMato cultivars Isola Aus, Aranka, Numerena, and others) were used as genotype reference. They were obtained from the Centre for Genetic Resources of the Netherlands (CGN). Tomato cultivars Isola, Aranka, Numerena, and others from South Africa, two Zaitian and twelve old Italian cultivars, were found to have a high level of heterogeneity within 25 local tomato cultivars from British, two varieties, and 6328 and VNT cherry were used as genotype reference. They are breeding lines from the Netherlands (CGN), Tomato cultivars Isola Aus, Aranka, Numerena, and others. The results obtained from this study suggest that the use of microsatellite markers can be useful for the identification of genetic diversity in tomato varieties. The results also indicate that the use of microsatellite markers can be useful for the identification of genetic diversity in tomato varieties.
The polymorphic alleles amplified by each pair of primers ranged from 7 to 22 (primer S189), with a mean of 13.5. The polymorphic alleles among the 88 distinct accessions. Ten primer pairs amplified a total of 135 SSR markers were screened out based on 16 pairs. Ten SSR markers were employed for analyses.

Yamane et al. (2009) assisted selection in eggplant breeding quantitative and qualitative trait mapping and for marker-assisted selection, and a useful resource for lines of S. melongena, the markers could be a useful resource for average polymorphic information content of 0.27 among eight pairs. 1,054 SSR markers were identified. The markers had an average polymorphic information content of 0.399 randomly selected primer pairs. 2,265 primer pairs were designed to select SSR markers and sequenced more than 1,400 clones. From these SSR markers constructed simple sequence repeat libraries in order to develop SSR primers.

Nunome et al. (2009) with landraces with landraces showed that black eggplants contained a considerable morphological and molecular diversity; it compared but commercial varieties, and in particular, F1 hybrids, displayed a reduced morphological and molecular diversity when compared with landraces as well as in six non-black control eggplants from different origins. The results showed that black eggplants contained a considerable morphological and molecular diversity. Molecular (ARF and SSR) diversity in a collection of 38 black molecular eggplant accessions, including commercial varieties and molecular accessions (ARF and SSR) measured the morphological and molecular variation among the Britten landraces.
bp. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was used to cluster the materials based on the genetic similarity of SSR bands. The similarity coefficient was calculated using the Jaccard index. The UPGMA cluster analysis revealed that all the materials were clustered into two major groups. The first group comprised the majority of the materials, while the second group contained a smaller number of materials.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The genetic relationships among the materials were also analyzed using a similarity coefficient matrix. The similarity coefficient matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The genetic relationships among the materials were also analyzed using a similarity coefficient matrix. The similarity coefficient matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.

The materials were also analyzed using a similarity coefficient matrix to determine the genetic relationships among the cultivars. The genetic similarity matrix was calculated using the Jaccard index, and the materials were then clustered using the UPGMA method. The cluster analysis revealed that the materials were grouped into two major clusters, with a smaller number of materials forming a separate cluster.
clearly separated from the other cultivated 

2.6% of the wild species’ intergenomic

clustering and the wild species’ 

Moreover, the fourth group can be further classified into 

which clustered into a separate and more distant 

value of 0.60 grouped all the 

S. torvum (0.26) and low average 

Additionally, S. melongena had high average 

an average of 0.69. The high diversity 

2.0151 ranged from 1.1307–5.310), respectively. 

information index and effective number of alleles were 0.41

all accessions measured by the allele’s genetic diversity.

values clustering in 

multiple polymorphic bands, two of which could be used for 

primer pairs (Em117, Em126, Em127) gave rise to 

6, with a mean of 3.6. Among 19 polymorphisms primers, 

detected alleles. The number of alleles per primer ranged from 2 

amplified, of which 19 showed polymorphism with a total of 66 

primers (Em117, Em126, Em127) gave rise to 

the USA cultivars forming a distinct group.

UPGMA clustering grouped the cultivars into five groups with
content (PIC) values ranged from 0.27 (SsrcAMS-405) to 0.67 (SsrcAMS-142). The polymorphism information
811 to five (SsrcAMS-142). The number of alleles per locus ranged one (SsrcAMS-
primer. The number of alleles per locus detected by six SSR primers, with an average of two alleles per
microsatellite (SSR) markers. A total of one to five alleles were
screening DNA from thirteen Capsicum cultivars using
Psael et al. (2011) assessed the genetic relation by
melanzana Rossa di Rotonda, cultivated in Italy.

conservation, improvement, and legal protection of the escupe
conservation, improvement, and legal protection of the escupe
others. These results provided additional information for
always morphologically and genetically distinguishable from the
cultivar with entries from Africa and Italy. These entries appeared in the
cultivar with entries from Africa and Italy. These entries appeared in the
cluster with entries from Africa and Italy. These entries appeared in the
cluster with entries from Africa and Italy. These entries appeared in the
the contrary, higher amount of variation was observed in the
the contrary, higher amount of variation was observed in the
American with limited rate of genetic variation was observed. On
dendrogram. A large cluster included several entries from South
sequence repeat (SSR) analyses were used to evaluate genetic
relationships among entries. Matrices of genetic similarities from
amplified fragment length polymorphism (AFLP) and simple
morphological traits and chloroplastic acid content. In addition,
sextrcted from variety Solanum chilense (entries from different
Sunseri et al. (2010) assessed genetic diversity among 70
concordant with taxonomic information.

majority of the cultivated eggplants were not clustered in ways
This study revealed the importance of guaranteeing the differentiation of chilli cultivars and the application for certification purposes.
Table 3.1: List of primordial genotypes used in present study

<table>
<thead>
<tr>
<th>No.</th>
<th>Name of Genotype</th>
<th>JB-08-08</th>
<th>JBG-09-08</th>
<th>JBG-19-08</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>JBG-1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>GP-149</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>GP-162</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>GP-175</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>GP-176</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>GP-181</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>GP-184</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>GP-191</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1: List of primordial genotypes used in present study

The seeds of primordial cultivars used for the present study were collected from the Vegetable Research Station, Junagadh Agricultural University, Junagadh. The experimental material comprised of sixteen genotypes of primordial which are listed in the experimental material. The seeds of primordial cultivars used for the present study were collected from the Vegetable Research Station, Junagadh Agricultural University, Junagadh.

3.1.1. Seed materials

3.1 EXPERIMENTAL MATERIAL

Junagadh during the year 2010-2011. College of Agriculture, Junagadh Agricultural University, Biotechnology Laboratory at Department of Agri-Botany, biochemical and molecular markers was conducted in different genotypes of primordial (Solanum melongena L) using the present investigation on "Molecular characterization of

MATERIALS AND METHODS

CHAPTER III
Plate 3.1 Photographic image of five cultivars of brinjal in present study.
Investigations are given in Table 3.2. The lists of chemicals used in present Technologics, USA. The lists of chemicals used in present MWC biotech Pvt Ltd, Germany, X-Integrated DNA used were obtained from Bangalore GeneI Pvt Ltd. Bangalore’s (SRL) etc. In case of some chemicals, molecular biological grade SISCO Research Lab. QMligenes, Chiti-Chem, CDH, Ran Kemp, from standard manufacturers like Sigma-Aldrich, Hi-Media, experiments were of either analytical grade or molecular grade. All the chemicals and the biochemicals used in the experiments were of either analytical grade or molecular grade.

3.1.4 Chemicals and Biochemicals

Bangalore, Karnataka

Synthetic primers used for various molecular marker

3.1.3 Primers

Stated above and air-dried before use. In an oven before use. All polywares were thoroughly cleaned as with tap water followed by distilled water. Finally, it was dried scrubbed and washed thoroughly with the detergent then rinsed such as Corning, BoroSil or Schott Duran. All glasswares were such as Corning, BoroSil or Schott Duran. All glasswares were used were of standard make.

3.1.2 Glasswares and Polywares
<table>
<thead>
<tr>
<th>Company</th>
<th>Chemical</th>
<th>Formula</th>
<th>MW</th>
<th>Regent name</th>
<th>ST. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>H3BO3</td>
<td>61.83</td>
<td>H2O</td>
<td>13.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>MgCl2, H2O</td>
<td>203.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>NaH2PO4</td>
<td>119.98</td>
<td></td>
<td>11.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>Na2HPO4</td>
<td>141.96</td>
<td></td>
<td>10.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>C2H3O2Na</td>
<td>82.03</td>
<td></td>
<td>9.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>C2H6O5</td>
<td>78.13</td>
<td></td>
<td>8.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>CH4N2O</td>
<td>60.06</td>
<td></td>
<td>7.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>(C6H9O)n</td>
<td>40000</td>
<td>Polyvinylpyrrolidone</td>
<td>6.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>NaCl</td>
<td>58.44</td>
<td>Sodium chloride</td>
<td>5.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>C19H24N8BrF</td>
<td>364.45</td>
<td>Bromide</td>
<td>3.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>C4H11N3O3</td>
<td>121.14</td>
<td>Tri-Buffer</td>
<td>2.</td>
</tr>
<tr>
<td>Amresco</td>
<td>Himmelia</td>
<td>C4H11N3O3.HCl</td>
<td>157.64</td>
<td>Tri-Sodium chloride</td>
<td>1.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Company</td>
<td>Chemical Name</td>
<td>Formular</td>
<td>MW</td>
<td>Regent name</td>
<td>ST. No.</td>
</tr>
<tr>
<td>---------</td>
<td>----------------</td>
<td>---------</td>
<td>----</td>
<td>-------------</td>
<td>-------</td>
</tr>
<tr>
<td>Himmelia</td>
<td>2.28.18</td>
<td>Per sulphate</td>
<td>21.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ammonium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycine</td>
<td>20.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Biss-carbamide</td>
<td>19.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>acrylicamide</td>
<td>18.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Xyline cyanal</td>
<td>17.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sucrose</td>
<td>16.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Glycitol</td>
<td>15.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Arabinose</td>
<td>14.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Boric acid</td>
<td>13.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hexahydrate</td>
<td>12.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Chloride</td>
<td>12.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Magnesium</td>
<td>12.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Monobasic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium phosphate</td>
<td>11.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Dibasic)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium phosphate</td>
<td>10.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium acetate</td>
<td>9.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mercuriothiol</td>
<td>8.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urea</td>
<td>7.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Polyvinylpyrrolidone</td>
<td>6.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium chloride</td>
<td>5.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tetra-Acetic acid</td>
<td>4.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ethanolamine</td>
<td>3.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Anmmonium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Compri Thyme</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ets-Buffer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Tri-Sodium chloride</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.2**: List of chemicals used for molecular and biochemical study.
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Regent name</th>
<th>Chemical formula</th>
<th>Company</th>
<th>MW*</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.</td>
<td>TEMED</td>
<td>C₅H₁₂N₂</td>
<td>Amresco</td>
<td>116.2</td>
</tr>
<tr>
<td>23.</td>
<td>Fast blue RR salt</td>
<td>C₁₅H₁₄ClN₃O₃</td>
<td>Himedia</td>
<td>387.90</td>
</tr>
<tr>
<td>24.</td>
<td>Alpha-naphthyl</td>
<td>C₁₂H₁₀O₂</td>
<td>Himedia</td>
<td>186.21</td>
</tr>
<tr>
<td>25.</td>
<td>acetate</td>
<td>C₁₂H₁₀Br₂O₄</td>
<td>Amresco</td>
<td>699.96</td>
</tr>
<tr>
<td>26.</td>
<td>Bromophenol blue</td>
<td>C₁₃H₉N₄O₅S</td>
<td>CDH</td>
<td>286.0</td>
</tr>
<tr>
<td>27.</td>
<td>blue R-250</td>
<td>C₁₄H₁₆N₂O₂</td>
<td>CDH</td>
<td>108.14</td>
</tr>
<tr>
<td>28.</td>
<td>p-Phenylenediamine</td>
<td>C₁₃H₁₇N₂O₄</td>
<td>CDH</td>
<td>244.30</td>
</tr>
<tr>
<td>29.</td>
<td>Ortho-dianisidine</td>
<td>C₁₃H₁₅N₂O₄</td>
<td>CDH</td>
<td>34.01</td>
</tr>
<tr>
<td>30.</td>
<td>Hydrogen peroxide</td>
<td>C₁₂H₂₅OSO₃Na</td>
<td>Amresco</td>
<td>288.38</td>
</tr>
<tr>
<td>31.</td>
<td>Sodium lauryl sulphate</td>
<td>K₂H₂PO₄</td>
<td>Sisco</td>
<td>136.09</td>
</tr>
<tr>
<td>32.</td>
<td>Potassium Phosphate (Dibasic)</td>
<td>P₂O₅</td>
<td>Sisco</td>
<td>178.18</td>
</tr>
<tr>
<td>33.</td>
<td>Phosphate (Monobasic)</td>
<td>CH₄O₂</td>
<td>HCL</td>
<td>60.05</td>
</tr>
<tr>
<td>34.</td>
<td>Acetic acid</td>
<td>C₂H₄O₂</td>
<td>HCL</td>
<td>32.04</td>
</tr>
<tr>
<td>35.</td>
<td>Methanol</td>
<td>CH₃OH</td>
<td>HCL</td>
<td>88.15</td>
</tr>
<tr>
<td>36.</td>
<td>Chloroform</td>
<td>C₂H₃Cl</td>
<td>HCL</td>
<td>119.38</td>
</tr>
<tr>
<td>37.</td>
<td>Hydrochloric acid</td>
<td>C₁₂H₅O₄</td>
<td>HCL</td>
<td>36.46</td>
</tr>
<tr>
<td>38.</td>
<td>Sodium hydroxide</td>
<td>NaOH</td>
<td>HCL</td>
<td>40.0</td>
</tr>
</tbody>
</table>
TABLE 3.3: List of instruments used in present study

The equipments and instruments which were used for present study are listed below in Table 3.3.

<table>
<thead>
<tr>
<th>Company name</th>
<th>Instrument</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Himedia, Genn, India</td>
<td>Laminar Air Flow</td>
<td>13</td>
</tr>
<tr>
<td>Zentrum Business, Area, India</td>
<td>Magnetic stirrer with hot plate</td>
<td>12</td>
</tr>
<tr>
<td>Remi Equipment Pvt, India</td>
<td>Water purification system</td>
<td>11</td>
</tr>
<tr>
<td>Millipore-Biix, India</td>
<td>Hot air oven</td>
<td>10</td>
</tr>
<tr>
<td>Agro tech, India</td>
<td>Transilluminator</td>
<td>9</td>
</tr>
<tr>
<td>Bangalore General Ltd, India</td>
<td>Microwave oven</td>
<td>8</td>
</tr>
<tr>
<td>Germany</td>
<td>Thermal cycle</td>
<td>7</td>
</tr>
<tr>
<td>Czech, Poland</td>
<td>Refrigerated centrifuge</td>
<td>6</td>
</tr>
<tr>
<td>M-Tronic, India</td>
<td>pH meter</td>
<td>5</td>
</tr>
<tr>
<td>University of K&quot;and, U.K (Vertical unit)</td>
<td>Gel documentation and analysis system</td>
<td>4</td>
</tr>
<tr>
<td>India, Japon (Vertical unit)</td>
<td>Gel electrophoresis unit</td>
<td>3</td>
</tr>
<tr>
<td>Bangalore General Ltd, India</td>
<td>Hot water bath</td>
<td>2</td>
</tr>
<tr>
<td>Bangalore General Ltd, India</td>
<td>Weighing balance</td>
<td>1</td>
</tr>
</tbody>
</table>

3.2 EXPERIMENTAL DETAILS

<table>
<thead>
<tr>
<th>Company name</th>
<th>Size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Himedia, Genn, India</td>
<td>Microbiopette of different Spectrophotometer</td>
</tr>
<tr>
<td>Zentrum Business, Area, India</td>
<td>Laminar Air Flow</td>
</tr>
<tr>
<td>Remi Equipment Pvt, India</td>
<td>Magnetic stirrer with hot plate</td>
</tr>
<tr>
<td>Millipore-Biix, India</td>
<td>Water purification system</td>
</tr>
<tr>
<td>Agro tech, India</td>
<td>Hot air oven</td>
</tr>
<tr>
<td>Bangalore General Ltd, India</td>
<td>Transilluminator</td>
</tr>
<tr>
<td>LG India Ltd, India</td>
<td>Microwave oven</td>
</tr>
<tr>
<td>Germany</td>
<td>Thermal cycle</td>
</tr>
<tr>
<td>Czech, Poland</td>
<td>Refrigerated centrifuge</td>
</tr>
<tr>
<td>M-Tronic, India</td>
<td>pH meter</td>
</tr>
<tr>
<td>University of K&quot;and, U.K (Vertical unit)</td>
<td>Gel documentation and analysis system</td>
</tr>
<tr>
<td>India, Japon (Vertical unit)</td>
<td>Gel electrophoresis unit</td>
</tr>
<tr>
<td>Bangalore General Ltd, India</td>
<td>Hot water bath</td>
</tr>
<tr>
<td>Bangalore General Ltd, India</td>
<td>Weighing balance</td>
</tr>
<tr>
<td>Czech, Poland</td>
<td></td>
</tr>
</tbody>
</table>
gel loading dye was added as described by Ageel et al. (1988). Supernatant was collected and 20 μl of
transferred it into centrifuge tube. The content was centrifuged at 10,000 rpm for 10 min. The
phosphate buffer (pH 7.2) with the help of mortar and pestle and

The 0.5g fresh tissue was extracted in 1 ml of 0.2M sodium

3.3.1.2 Extration of Protein

and finally make up the volume 200ml with distilled water.

Adjust the pH 7.2 by adding the solution-A into solution-B

100ml distilled water.

Sodium phosphate buffer (pH 7.2)

100ml distilled water.

Sodium dihydrogen orthophosphate in

Sodium dihydrogen orthophosphate in

3.3.1 Preparation of 0.2M Sodium Phosphate buffer (pH 7.2)

3.4.

Native Polycrylamide Gel Electrophoresis are given in Table

and Mannikam, 1997. The stock solutions/buffer needed for

separation is based on the size of protein molecules (sedimentation

The protein complex move towards the anode and the

3.3.1 Protein Problime (Native PAGE)

After 12 and 18 days after germination.

For the study of biochemical parameters like isozymes and

3.3 Biochemical Characterization

For protein and isozymes analysis as well as molecular analyses.

The experiment was conducted with following details.
3.3.1.3 Separating gel (10%)  
Ten ml of Acrylamide from stock solution and 8.0 ml of 1.5 M Tris buffer was added in the beaker, followed by 12 ml of distilled water. The content was degassed up to five minutes. After degassing, 150 µl of freshly prepared ammonium per sulphate was added followed by 50 µl TEMED. The flask was shaken well and contents transferred quickly into the PAGE unit chamber between the glass plates. The gel was left for polymerization (15 – 20 minutes).

3.3.1.4 Stacking gel (4%)  
Take 1.35 ml Acrylamide solution into a beaker followed by adding of 2.5 ml Tris buffer 0.5 M and 6.0 ml distilled water. After degassing for 10 minute, 50 µl Ammonium per sulphate and 30 µl TEMED were added and mixed well. The solution was poured in the PAGE unit chamber containing polymerized separating gel. The comb was placed in the stacking gel and allowed to set for 20 minutes. The comb was removed after complete polymerization without disturbing the shape of the well.

3.3.1.5 Loading of sample:  
Load 35 µl of sample into each well with the help of micropipette. Formation of bubbles was avoided during loading. The electrophoresis was carried out on vertical PAGE. After loading the sample, electrode buffer was poured to the electrophoresis unit. The electrophoresis unit was connected with the power supply. The current was turned on allowing 30 mA 120 volts for initial 20 minutes until the sample travels through the stacking gel. Then current was increased up to 50 mA till the tracking dye reach to the bottom. (Sadasivam and Manikam, 1992).
reaction products. After sufficient incubation period, the reaction
in the gel will be visualized due to the appearance of colored
and Manickam, 1992). The zones where the enzymes are located
were incubated in the respective substrate solution (sodium
210°C temperatures. Immediately after electrophoresis, the gels
Native polyacrylamide gel electrophoresis was conducted at

3.3.2 Isoenzymes electrophoresis

volume was adjusted to 200 ml.
respectively. Finally, add 28.0 ml of A and 72.0 ml of B and final
H2O in 100 ml (separately and desalted as A and B.
phosphate (53.65 g of Na2HPO4•7H2O or 71.7 g of Na2HPO4•12
phosphate) (27.8 g in 100 ml) and 0.2 M solution of dibasic sodium
Prepare 0.2 M solution of monobasic sodium phosphate

3.3.2 Preparation of phosphate buffer:

(Ph - 7.2).

3.3.2.1 Enzyme estimation

Enzyme estimation is done in cold phosphate buffer

3.3.2.2 Isoenzyme

of the different protein bands was recorded manually.
continued until background was colorless. The relative mobility
was determined by putting in destaining solution. The process was
was desalted by putting in desalting solution. The process was
staining and this was continued for at least one hour. The gel
staining in a tray, The tray was periodically shaken for uniform
plates, immersed in bromophenol blue staining solution
off. The gel was gently removed from the space between the
after complete separation of molecules, power supply was turned
When the tracking dye reached the end of the running gel

3.3.1.6 Removing the Gel
data. The gel was incubated in the following solutions:

Enzyme source. Native PAGE or the sample extracts was carried
at 12,000 rpm for 15 minutes at 4°C. Supernatant is used as an
phosphate buffer (pH 7.2). The homogenate was centrifuged at
Brinjal seedlings (0.5 & 0.2 M) were grounded in cold 0.2 M NV|

3.3.2.5 Peroxidase

10:10:2:1 (Desborough et al., 1967).

Mechanistic: distilled water: acetic acid: ethanol alcohol in the ratio
enzymatic reactions were stopped by adding a mixture of

<table>
<thead>
<tr>
<th>Quantity</th>
<th>Reagents</th>
</tr>
</thead>
<tbody>
<tr>
<td>200ml</td>
<td>Distilled water</td>
</tr>
<tr>
<td>0.03%</td>
<td>4-naphthyl acetate</td>
</tr>
<tr>
<td>0.2%</td>
<td>Fast blue RR salt</td>
</tr>
<tr>
<td>1.1%</td>
<td>Disodium hydrogen phosphate</td>
</tr>
<tr>
<td>2.8%</td>
<td>Sodium dihydrogen phosphate</td>
</tr>
</tbody>
</table>

The following solutions:

37°C for 20-30 min, preferably in dark. Gel was incubated in
the gel is incubated in a solution given below at
an enzyme source. Native PAGE of the sample extracts was
carried out. The gel is incubated in a solution given below at

3.3.2.4 Esterase

Isoenzymes are described as under:

Reference. Enzyme extraction and staining procedure for various
zymogram. The relative position of each
photography of zymogram. The relative position of each
was stopped by adding appropriate stop solution followed by

Materials and Methods
using 50% ethanol.

Reddish brown bands were visible in the gel. Fix the gel

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1 M Acetate buffer (pH 5.0)</td>
<td>10 ml</td>
</tr>
<tr>
<td>0.5 ml</td>
<td>Magnesium chloride</td>
</tr>
<tr>
<td>50 ml</td>
<td>Sodium chloride</td>
</tr>
<tr>
<td>50 ml</td>
<td>Fast Blue RR Salt</td>
</tr>
<tr>
<td>50 ml</td>
<td>α-Naphthyl phosphate</td>
</tr>
</tbody>
</table>

min, in dark conditions.

The sample material was homogenized in 5-fold volume of

3.3.2.6 Acid Phosphatase

Immersion of the gel in 7% acetic acid solution for 10 min (van

Hydrogen peroxide (3%) was carefully added drop by drop

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>100 ml</td>
</tr>
<tr>
<td>Methanol</td>
<td>40 ml</td>
</tr>
<tr>
<td>Acetic acid</td>
<td>10 ml</td>
</tr>
<tr>
<td>2.0% O-dianisidine</td>
<td>0.8 ml</td>
</tr>
</tbody>
</table>
are given in Table 3.5.

Procedure for preparation of various stock solutions and buffers

3.4.1 Preparation of stock solutions for reagents and buffers

(1987) with minor modifications, carried out by CTAB method as described by Doyle and Doyle.

The various stock solutions were prepared well in advance as per Sambrook et al. (1989) and were used for extraction of DNA from binural leaves. The composition and extraction were described in different binural plants grown in pots. DNA extraction will be performed in 0.1 M potassium phosphate buffer (pH 7.0) followed by addition of 10 mM catechol (MW-110 E/mole) in the same buffer. After staining the gel is washed with millipore water and prepared in 0.1 M potassium phosphate buffer followed by equilibration for 30 min in 0.1% p-phenylenediamine. The enzyme extraction, preparation, and electrophoresis are same as peroxidase isoamylase. After electrophoresis, the gel is stained with 0.05% Coomassie Brilliant Blue R-250 and destained with 10% acetic acid.

3.4.1 DNA Extraction

3.4 MOLECULAR CHARACTERIZATION

Photographed for further analyses (Van Loon, 1971).

Total genomic DNA was isolated from young leaves of
<table>
<thead>
<tr>
<th>Method of Preparation</th>
<th>Solution</th>
<th>No.</th>
<th>St.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ISOAMYL ALCOHOL:</td>
<td>100 ml</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>CHLOROFORM:</td>
<td>70% ETHANOL</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>4 ml of CHLOROFORM and 4 ml of ISOAMYL ALCOHOL</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and stored at 4°C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and dispersed to reagent bottle at 70°C or 80°C</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>diluted with distilled water</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and pH adjusted to 8.0 by adding 0.2 M HCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.0 M THIS HCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.2 M EDTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>18.60 μl of sodium salt was dissolved in 80 ml of distilled water.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20.2 and 50 ml of distilled water was added</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0 M NaCl</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2× CTAB</td>
<td>4.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.8 PVP was taken into beaker and mixed well and dispersed to reagent bottle at 4°C.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>and stored at room temperature.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sterilized by autoclaving.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>was dispersed to reagent bottle and adjusted to 100 ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Its final volume was 100 ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>sterilized by autoclaving.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>was dispersed to reagent bottle and adjusted to 100 ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Its final volume was 100 ml.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.5: Reagents used for the preparation of stock solution**
<table>
<thead>
<tr>
<th><strong>Method of Preparation</strong></th>
<th><strong>Solution</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><strong>No. 13</strong></td>
</tr>
<tr>
<td></td>
<td>10 mg/ml</td>
</tr>
<tr>
<td></td>
<td>1 M TrisCl (pH 7.4)</td>
</tr>
<tr>
<td></td>
<td>250 mg of bromophenol blue, 250 mg dye</td>
</tr>
<tr>
<td></td>
<td>12-20°C. Dispense into aliquots and store at room temperature. Ph was adjusted by 0.1 ml of 1 M TrisCl (pH 7.4).</td>
</tr>
<tr>
<td></td>
<td>100 ml of distilled water were added in 100 ml of distilled water of xylene cyanol FF and 30% glycerol.</td>
</tr>
<tr>
<td></td>
<td>adjusted to 8.0.</td>
</tr>
<tr>
<td></td>
<td>20.22 g sodium acetate hydrate</td>
</tr>
<tr>
<td></td>
<td>2 ml of 0.25 M EDTA, 5 ml of this base, 27.5 ml of 1× TBE buffer</td>
</tr>
<tr>
<td></td>
<td>2 ml of 0.5 M HCl</td>
</tr>
<tr>
<td></td>
<td>4 ml of this HCl (pH 8.0)</td>
</tr>
<tr>
<td></td>
<td>10 ml of 1× TBS</td>
</tr>
<tr>
<td></td>
<td>10 ml of 1× TBS</td>
</tr>
<tr>
<td></td>
<td>1 ml of 10× TBS</td>
</tr>
</tbody>
</table>

Table 3.5: Contd.

...
For 15 minutes to ensure emulsification of the phase.
Isopropanol (2:5:2:4:1) was added and mixed by inversion
Subsequent tube and one volume of phenol: Chloroform:
One ml aqueous phase was transferred to another fresh
(25°C).
Spin at 12,000 rpm for 10 minutes at room temperature
Swirling.
Incubate for one hour at 65°C in water bath with gentle
Gentle tubes.
Transfered homogenized material in capped polypropylene
extraction buffer (2X CTAB)
pre-warmed (1:5 ml, 65°C) DNA isolation buffer (2X CTAB
Weighed 0.5 to 1.0 g of leaf tissue and ground in

3.4.1.3 DNA Extraction Procedure:

<table>
<thead>
<tr>
<th>Amount per 100 ml</th>
<th>Concentration</th>
<th>Final Stock Solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 ml</td>
<td>24 ml</td>
<td>Water</td>
</tr>
<tr>
<td>4 ml</td>
<td>4%</td>
<td>APD</td>
</tr>
<tr>
<td>4 ml</td>
<td>0.5 M</td>
<td>Urea</td>
</tr>
<tr>
<td>20 ml</td>
<td>1 M</td>
<td>IM</td>
</tr>
<tr>
<td>28 ml</td>
<td>2%</td>
<td>Stock Solution</td>
</tr>
</tbody>
</table>

20 ml solution and the buffer pre-warm to 65°C.
20 ml solution and the buffer pre-warm to 65°C.
Into a clean 50-ml tube and add 40 ml of p-mercaptothanol per
H2O. Just before use, enough volume to be used was aliquoted
following components and adjusting to 100 ml with ultrapure
The 2X CTAB extraction buffer was prepared by using

3.4.1.2 Preparation of extraction buffer for DNA extraction

Materials and Methods
Kept in 30 minutes at room temperature without agitation.

17. Each pellet was re-dissolved in 100 µl of TE buffer by gentle agitation.

18. The tubes were inverted and drained on a paper towel. Then, each pellet and was kept for 10 minutes at 4°C.

19. After removing supernatant, 300 µl of 70% alcohol was added in pellet and was kept for 10 minutes at 4°C.

20. Equal volume of ice-cold isopropanol was added to aqueous phase to precipitate DNA.

21. Repeat the steps 8 to 10 once again.

22. Aqueous phase was transferred to another fresh tube.

23. Spin at 12,000 rpm for 10 minutes at room temperature.

24. Ensure emulsification of the phase.

25. One ml aqueous phase was transferred to another Eppendorf tube.

26. Spin at 12,000 rpm for 10 minutes at room temperature.
Bromide to 1X TBE buffer (100 ml) containing (0.5 M Tris-HCl, 8 M of EDTA) was added following steps.

The integrity of DNA was judged on agarose gel analyses in

3.4.1.5 Gel Analysis

use

9. The DNA was re-dissolved in 100 ml of TE buffer for further

for 30 minutes.

8. The pellet was washed with 300 ml 70% ethanol and air dried

5 minutes at 4°C.

7. To pellet the DNA, the tubes were centrifuged at 5000 rpm for

absolute ethanol.

6. The DNA was re-precipitated by adding double the quantity of

interface.

5. Supernatant was taken, avoiding the whitish layer at

4. Spin at 12,000 rpm for 10 minutes at 4°C.

for 10 minutes till an emulsion was formed.

3. Ten ml of 3M sodium acetate was added and mixed thoroughly

minutes.

2. It was mixed thoroughly and incubated at 37°C for 30

preparation.

1. One ml of RNase was added to 100 ml of crude DNA

out in following steps.

To get RNA free DNA sample, the purification was carried

3.4.1.4 Purification of DNA
is concentration, 6. If the concentration of RAPD primer OPA-12 is 1.721 µmol, then adding 17.21 µl of deionized water equal to 1000 µmol.

3. RAPD DNA (RAPD) - Inter Simple Sequence Repeat (ISSR) and Simple Sequence Repeat (SSR) were used. Chemicals for molecular marker techniques such as Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR) and Simple Sequence Repeat (SSR) were used. Chemicals for molecular marker techniques such as Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR) and Simple Sequence Repeat (SSR) were used. Chemicals for molecular marker techniques such as Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeat (ISSR) and Simple Sequence Repeat (SSR) were used.

Materials and Methods

3.4.2 PCR based molecular markers

Finally, concentration of 100 ng/µl in the buffer was calculated by the software. The concentration of DNA was diluted to 10 ng/µl in the buffer. The concentration of DNA was measured using a spectrophotometer (Thermo Scientific, USA) and Nanodrop.

3.4.1.6 Quantification of DNA

Isolated DNA. Lambda phage DNA indicates high molecular weight of DNA. Presence of single compact band at the corresponding band of DNA. 5. Visualize the gel under UV light.

4. Run the gel at 50 V for one hour. Control in the adjacent well.

3. Load a known amount of uncult Lamdba phage DNA as a promophenol blue.

2. Load 20 µl DNA sample properly mixed with 3 µl
The following Operon series of RAPD primers were used for PCR protocol.

(a) Primer (25 p moles/µl)
(b) dNTPs (dATP, dCTP, dGTP and dTTP)
(c) Taq DNA polymerase
(d) PCR buffer (10X)

These reagents were followed for RAPD-PCR amplification of DNA.

RAPD-PCR Components

Technologies Inc., USA.

Using random 10-mer oligonucleotide primers from Operon
through DNA marker (RAPD), the RAPD assays were performed
modifications was followed for molecular characterization
The method given by Singh et al. (2002) with minor

3.4.2.1 Randomly Amplified Polymorphic DNA (RAPD)

Template DNA was added separately in each tube.

Appropriate amount of cocktail was dispensed to each tube and
one reaction with total number of samples. Later, the
reactions were combined in one tube multiplying the volume for
cocktail was prepared where constituents common to all the
to avoid pipetting error in measuring small volumes, a

techniques.

solution was used for PCR amplification for various molecular
water gave a final concentration of 10 µmoles/µl. This working
of stock (1000 µmoles/µl) and 99 µl of distilled sterile deionized
The reagents used for electrophoresis or amplified RAPD

Chemicals used

Gel agarose 1.5%

The amplified products of RAPD were analyzed using 1.5% agarose gel.

(4) Electrophoresis of Amplified Product

<table>
<thead>
<tr>
<th>Duration</th>
<th>Temperature (°C)</th>
<th>Step</th>
<th>ST. NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 min</td>
<td>72</td>
<td>Initial Denaturation</td>
<td>4</td>
</tr>
<tr>
<td>1 min</td>
<td>36</td>
<td>Annealing</td>
<td>3</td>
</tr>
<tr>
<td>1 min</td>
<td>94</td>
<td>Extension</td>
<td>2</td>
</tr>
<tr>
<td>4 min</td>
<td>94</td>
<td>Extension</td>
<td>1</td>
</tr>
<tr>
<td>Repeat the steps 2 to 4 for 40 times</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 min</td>
<td>72</td>
<td>Hold</td>
<td>6</td>
</tr>
</tbody>
</table>

PCR conditions for RAPD

The conditions for RAPD amplification in the Thermal Cycle as follows:

(1) RAPD-PCR conditions:

- 3 min at 94°C
- 40 cycles:
  - 1 min at 94°C
  - 1 min at 36°C
  - 1 min at 72°C
- 7 min at 72°C

---
Primers were used for study. (Table 3.7)

Following UBC (University of British Columbia, Canada)

(1) List of ISSR Primers

PCR Protocol

(d) Primer (25 p moles/1)

(e) dNTPs (dATP, dCTP, dGTP and dTTP)

(b) Taq DNA Polymerase

(a) PCR Buffer (10×)

The reagents used for ISSR-PCR amplification of DNA were

ISSR-PCR Components

British Columbia (Canada) primers.

The genomic DNA was amplified using UBC (University of

The PCR reactions for ISSR were carried according to

3.4.2.2 Inter Simple Sequence Repeats (ISSR)

...continued...
## Table 3.7 List of ISSR primers used in the present study

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Primer</th>
<th>GC %</th>
<th>GC %</th>
<th>Sequence 5'-3'</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>UBC 807</td>
<td>47</td>
<td>47</td>
<td>AGAGAGAGAGAGAGACCT</td>
</tr>
<tr>
<td>2</td>
<td>UBC 810</td>
<td>47</td>
<td>47</td>
<td>AGAGAGAGAGAGAGACCTT</td>
</tr>
<tr>
<td>3</td>
<td>UBC 813</td>
<td>47</td>
<td>47</td>
<td>GTCTCTCTCTCTCTGTA</td>
</tr>
<tr>
<td>4</td>
<td>UBC 814</td>
<td>47</td>
<td>47</td>
<td>GTCTCTCTCTCTCTGTA</td>
</tr>
<tr>
<td>5</td>
<td>UBC 815</td>
<td>53</td>
<td>53</td>
<td>GTCTCTCTCTCTCTGTA</td>
</tr>
<tr>
<td>6</td>
<td>UBC 815</td>
<td>53</td>
<td>53</td>
<td>GTCTCTCTCTCTCTGTA</td>
</tr>
<tr>
<td>7</td>
<td>UBC 822</td>
<td>47</td>
<td>47</td>
<td>GTCTCTCTCTCTCTGTA</td>
</tr>
<tr>
<td>8</td>
<td>UBC 823</td>
<td>47</td>
<td>47</td>
<td>GTCTCTCTCTCTCTGTA</td>
</tr>
<tr>
<td>9</td>
<td>UBC 824</td>
<td>47</td>
<td>47</td>
<td>GTCTCTCTCTCTCTGTA</td>
</tr>
<tr>
<td>10</td>
<td>UBC 866</td>
<td>67</td>
<td>67</td>
<td>ACTCTCTCTCTCTGTAACCA</td>
</tr>
<tr>
<td>11</td>
<td>UBC 900</td>
<td>48</td>
<td>48</td>
<td>ACTCTCTCTCTCTGTAACCA</td>
</tr>
</tbody>
</table>

(ii) Preparation of reaction mixture

The reaction mixture for ISSR-PCR was consisted of the following reagents:

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Reagent</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PCR buffer (10X)</td>
<td>2.5 μl</td>
</tr>
<tr>
<td>2</td>
<td>MgCl2 (2.5 mM)</td>
<td>1.5 μl</td>
</tr>
<tr>
<td>3</td>
<td>Taq polymerase (3 U/μl)</td>
<td>0.5 μl</td>
</tr>
<tr>
<td>4</td>
<td>dNTPs mix (2.5 μM each)</td>
<td>1.5 μl</td>
</tr>
<tr>
<td>5</td>
<td>Primer (25 pmole/μl)</td>
<td>2.5 μl</td>
</tr>
<tr>
<td>6</td>
<td>Template DNA (50 ng/μl)</td>
<td>2.0 μl</td>
</tr>
<tr>
<td></td>
<td>Millipore Sterile distilled water</td>
<td>10.5 μl</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>20.0 μl</td>
</tr>
</tbody>
</table>

The genomic DNA fingerprinting by ISSR was subjected to Amplification was tested for 10 UBC series primers. All the PCR reactions were carried out in 0.2 ml capacity thin walled PCR tubes. As per the above cocktail, Millipore sterilized water was added first followed by addition of PCR mastermix (Bangalore Genei Pvt. Ltd.), primer in sequence finally by tapping against the tube followed by a short spinning (~3,000 rpm for 30 seconds). The tubes were then placed in the Thermal Cycler for cyclic amplification.
3.4.2.3 Simple Sequence Repeats (SSR)

Agarose gel same as RAPD.

The amplified products of ISSR were analyzed using 1.5 %

<table>
<thead>
<tr>
<th>Step</th>
<th>Duration</th>
<th>Temperature (°C)</th>
<th>PCR condition for ISSR</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30 sec</td>
<td>94</td>
<td>Initial</td>
</tr>
<tr>
<td>2</td>
<td>45 sec</td>
<td>55</td>
<td>Denaturation</td>
</tr>
<tr>
<td>3</td>
<td>2 min</td>
<td>72</td>
<td>Extension</td>
</tr>
<tr>
<td>4</td>
<td>2 (35 times to step 4)</td>
<td>72</td>
<td>Extension</td>
</tr>
<tr>
<td>5</td>
<td>0 min</td>
<td>2</td>
<td>Final extension</td>
</tr>
<tr>
<td>6</td>
<td>Hold</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The thermal cycle was set to following cyclic condition for

Materials and Methods
(i) PCR Protocol

(a) Forward and reverse primer (25 p moles/µl) each
(b) dNTPs (dATP, dCTP, dGTP and dTTP)
(c) Taq DNA polymerase
(d) PCR buffer (10X)

The reagents used for SSR-PCR amplification of DNA were as follows:

SSR-PCR Components

Materials and Methods
<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Markers</th>
<th>Sequence 5’ – 3’</th>
<th>Tm (°C)</th>
<th>GC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>smSSR09</td>
<td>F</td>
<td>58.3</td>
<td>50 %</td>
</tr>
<tr>
<td>2</td>
<td>smSSR11</td>
<td>R</td>
<td>59.0</td>
<td>40 %</td>
</tr>
<tr>
<td>3</td>
<td>smSSR12</td>
<td>F</td>
<td>58.4</td>
<td>40 %</td>
</tr>
<tr>
<td>4</td>
<td>smSSR13</td>
<td>R</td>
<td>58.7</td>
<td>44 %</td>
</tr>
<tr>
<td>5</td>
<td>smSSR14</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>smSSR15</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>smSSR16</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>smSSR17</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>BEAMS17</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>BEAMS15</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>BEAMS28</td>
<td>F</td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>BEAMS48</td>
<td>R</td>
<td></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>BEEMS37</td>
<td>F</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.8: List of SSR primers used in present study
trans-illuminator. RAPD, ISSR and SSR bands were designated. The gel was taken by a Gel Documentation System, under UV light. In order to score and preserve banding pattern, photographs were taken at the same time.

### 3.5 Statistical Analysis

The amplified products of SSR were analyzed using 2.5%

\[ \text{Electrophoresis of Amplified Product:} \]

<table>
<thead>
<tr>
<th>Duration (°C)</th>
<th>Temp.</th>
<th>Steps</th>
<th>St. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 min</td>
<td>94</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1 min</td>
<td>72</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3 min</td>
<td>72</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4 min</td>
<td>72</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5 min</td>
<td>72</td>
<td>5</td>
<td>Hold</td>
</tr>
<tr>
<td>6 min</td>
<td>72</td>
<td>6</td>
<td>Final extension</td>
</tr>
</tbody>
</table>

Repeat the steps 2 to 4 for 36 times.

### PCR Conditions for SSR

The thermal cycler was set to following cycle condition for SSR amplification.

The thermal cycler was set to following cycle condition for SSR amplification.
Clustering is the equal rate of evolution along all dendrograms. The assumption underlying the use of UPGMA (unweighted pair-group method with arithmetic averaging) was produced and a dendrogram constructed using a tree matrix was produced and a dendrogram constructed using hierarchical, and nested clustering methods (clustering program, matrix was entered into SAHN (sequential, agglomerative, of similarity or dissimilarity coefficients, The resultant similarity matrix of 0 and 1 acts as the input, and the output is a matrix A matrix of 0 and 1 acts as the input, and the output is a matrix the basis for similarity analysis among various bacterial genotypes. absence (0) or presence (1) state of a RAPD marker was used as a coefficient for qualitative data. The qualitative nature of the program for computing a variety of similarities and dissimilarities was used by the SIMQUAL (similarity coefficient) program with Jaccard's similarity coefficient. SIMQUAL is a program developed by R. J. Rohlf, Personal Computers, Exeter Software, developed by R. J. Rohlf, Numerical Taxonomy and Multivariate Analysis System for the data matrix was read by NTSYS-pc version 2.2.2.

using NTYSYPC version 2.02 (Rohlf, 1994).

The data matrix was entered into MS-Excel data sheet and subsequently analyzed. Corresponding band among the genotypes. The data were Clear and distinct bands amplified by RAPD, ISSR and SSR bands were not scored. Band was scored as '1', and its absence as '0'. Partially visible reference to molecular weight of marker. The presence of each fragment from the well was translated to molecular size with to estimate the molecular size. The distance run by amplified loaded simultaneously with primer products in the gel was used on the basis of their molecular size (length of polynucleotide.
Referred as follows (Rohlf, 1994):

The degree of fit can be analyzed using the Mantel test statistic. The degree of fit is a measure of goodness of fit for a cluster analysis or a cluster matrix, which is correlated to the Mantel test statistic and in standardized units, as the phenetic correlation coefficient is positively correlated.

\[ \text{V}_i = \text{off-diagonal elements of original tree matrix} \]
\[ \text{X}_i = \text{off-diagonal elements of phenetic value matrix} \]
\[ f > 1 \]
\[ \frac{\text{V}_i \times \text{X}_i}{n} \]

3.5.1. The test criterion of Mantel test

Statistic (Z) (Rohlf, 1994). The program MWCMP (matrix comparison program) was done by the MWCMP program which compares the original tree matrix and computes the correlation against the original tree matrix. This type of phenetic correlation was compared with the original tree matrix for goodness of fit of the COPH (phenetic values). The phenetic correlation calculated using the tree matrix produced by SAVHN to calculate the phenetic values of similarity or dissimilarity by the program was made for the existence of true clusters. This was done by clusters. Where clusters exist in the data, or not, a check of whether there are true clusters in the data, or not, in a check.

In graphic mode:

Cluster methods create clusters of the data. From the output tree file of SAVHN by TREE (tree display) program branches, Dendrogram of phenetic quality were produced.
\[ n = \text{the number of elements of the matrices} \]
\[ V_{1*} = \text{off-diagonal elements of original tree matrix} \]
\[ X_{1*} = \text{off-diagonal elements of phenetic value matrix} \]

\[ \text{Distance migrated by the dye marker} = \frac{R_{m}}{\text{Distance migrated by the enzyme band}} \]

Equation (Escurea and Peiris, 2001).

Each genotype was tested using the following equation (Homberg, 1993).

\[ \text{Relative mobility (RM)} \]

The relative mobility (RM) of each band was measured in each genotype. For every genotype tested, the following equation was used:

\[ 3.5^2 \text{ RM (Relative Mobility)} = \text{Value} \]

\[ \text{Where } P_i \text{ is the frequency of } i \text{ allele for marker } i, \text{ and} \]

\[ \text{PIC} = \frac{1}{u} \sum_{i=1}^{k} \left( 1 - \frac{\sum_{j=1}^{n} d_{ij}^2}{4} \right) \]

\[ \text{Allele frequency (Anderson et al., 1993)} \]

The PIC value for each locus was calculated on the basis of the following table:

<table>
<thead>
<tr>
<th>Degree of Fit</th>
<th>PIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very poor fit</td>
<td>\text{r}^2 &gt; 0.7</td>
</tr>
<tr>
<td>Poor fit</td>
<td>0.7</td>
</tr>
<tr>
<td>Good fit</td>
<td>0.8</td>
</tr>
<tr>
<td>Very good fit</td>
<td>0.9</td>
</tr>
<tr>
<td>Degree of fit</td>
<td>Level</td>
</tr>
</tbody>
</table>
Results & Discussion

Chapter IV
CBF-1 was found only in 0.60 Rm value.
PLR-1 and in rest of the genotypes it was absent. Genotype
Rm value of 0.25 was present only in two genotypes JBER-1 and
the binual genotypes except CBF-1 and CP-162. A marker of
JBER-08-08, JBER-08-10-1 were polymorphic. The band of Rm value
the genotypes except CBF-1, CP-1. JBER-08-08, JBER-08-10-1
were found in all 0.742 and 0.840 (Plate 4.1 and Table 4.1). All the eight bands
having Rm values of 0.242, 0.295, 0.365, 0.458, 0.525, 0.604,
Protein profile of binual genotypes developed eight bands

4.1.1.1 Protein profile at 7 DAG

The study of genetic diversity among them.
All 16 binual genotypes were tested in Native-PAGE for

4.1.1 Native PAGE (Protein)

4.1 Biochemical Markers

4.2 Molecular markers (RAPD, ISSR, SSR)

4.3 Pooled study of all biochemical and molecular markers

below in two parts. and molecular marker analysis are presented and discussed.

molecular marker analysis. The results obtained on biochemical
solanum melongena L. (Genotypes using biochemical and
study the genetic diversity analysis among sixteen binual
The present investigation was undertaken with a view to

RESULTS AND DISCUSSION

CHAPTER 4
Plate 4.1: Protein profile generated using polyacrylamide gel electrophoresis at 7 and 14 days after germination.
<table>
<thead>
<tr>
<th>Band Number (Rm Value)</th>
<th>0.840</th>
<th>0.742</th>
<th>0.604</th>
<th>0.525</th>
<th>0.458</th>
<th>0.365</th>
<th>0.242</th>
<th>Genotypes</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP-184</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-181</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-176</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-175</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-162</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-149</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-144</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBG-08-10-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBG-08-08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBG-13-2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBG-06-08</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB-I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBG-02-11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBI-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PII-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBG-1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 4.1: Rm values of banding pattern of protein profiling from 7 DAG brinal seedlings.
and GEL-1. The cluster-II split into two subclusters that is a
similarity. The cluster-I consists of mainly two genotypes GP-162
partitioned into two main clusters I and II with about 21%
dendrogram constructed revealed that 16 binarial genotypes were
using Jaccard's similarity coefficient (Table 4.3). The
cluster analysis was carried out by the UPGMA method
about 0.925.
Poly-morphic. Seven and fourteen days PIC value was found
different DAG (7 and 14 days) in which all the bands were
A total of 16 bands were generated by protein profile at
Genotypes showed its absence.
Presence of bands at Rm 0.711, while all the remaining
values of 0.603 were found in all
markers of Rm value 0.603 were found in all
1.44, GP-175, GP-176 and GP-184, respectively. Band of Rm
values of 0.603, 0.479, 0.603, 0.711 and 0.818 (Table 4.1, Table 4.2). Only
bands at all Rm value, Bands of Rm value 0.958 and 0.135 were
the genotypes viz., JBER-1, PLR-I, GBD-I, JBER-08-10-1, GP-
60-08, JBER-08-10-1, GP-144, GP-175, GP-176 and GP-184
shown by nine genotypes namely JBER-1, PLR-I, GBD-I, JBER-
three genotypes viz., JBER-1, PLR-I, and GP-175 were showing
protein profile of binarial genotypes developed eight
4.1.2 Protein profile at 14 DAG
<table>
<thead>
<tr>
<th>Genotypes</th>
<th>GP-114</th>
<th>GP-149</th>
<th>GP-162</th>
<th>GP-175</th>
<th>GP-176</th>
<th>GP-181</th>
<th>GP-184</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Band Number (Rm Value)

Table 4.2: Rm Values of banding pattern of protein profiling from 14 DAG brinjal seedlings.
Table 4.3: Jackard's similarity coefficient of 16 binary genotypes based on protein profiling.
Fig. 4.1: Dendrogram depicting the genetic relationship among 16 Brinjal genotypes based on 7 DAG and 14 DAG protein profile data.
and C comprising 7 and 7 genotypes, respectively in Capsicum.

while Akbar et al. (2010) shown Group of three main clusters viz: A, B and C comprising 7, 5 and 7 genotypes, respectively. Our dendrogram constructed classified the sixteen binua study. Our dendrogram constructed classified the sixteen binua

result (21-100%).

percent which were different from present result (21-100%).

variant from 10-22.22 percent.

proteins of five selected experimental genera of Solanaceae were observed in present finding. The similarity index [S.1] calculated after calculations of the Solanum species. The maximum eight protein bands were obtained in Solanum melongena while investigations on Solanum batata, Solanum acaia and Solanum melongena while investigations on Capsicum annuum. They obtained maximum five protein bands in case of Capsicum.

Similar findings were also reported by Bhar and Kudzia (2011).

diverse in BII group.

Genotypes GP-175, JBR-02-11 and JBRG-13-2 were found to be

JBGR-1 and PLR-1 were found closely similar with 100% similarity.

six genotypes, GP-181 and GP-184, JBRG-06-08 and GP-149, and

while cluster BII was split into five genotypes (JBRG-13-2), while cluster was split into

GP-176 showed nearly 66% similarity (cluster A II). Sub-cluster B

44 closely similar with 100% similarity (cluster A II). Sub-cluster B

and B sub-cluster A consists of five genotypes: GOB-1, GP-144.
Polyphosphate Phosphatase, the Puromycinase and Barbiturate oxidase, and Peroxidase were used for studying the variation through establishing genetic purity of seeds, etc. In the present experiment, 16 isoenzymes were useful in evolutionary study, gene activity and as an IZ2 DAG.
Plate 4.2: Polyacrylamide gel electrophoresis of Esterase and Peroxidase isoenzyme at 12 days after germination.
<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP-184</td>
<td>16</td>
</tr>
<tr>
<td>GP-181</td>
<td>15</td>
</tr>
<tr>
<td>GP-176</td>
<td>14</td>
</tr>
<tr>
<td>GP-175</td>
<td>13</td>
</tr>
<tr>
<td>GP-162</td>
<td>12</td>
</tr>
<tr>
<td>GP-149</td>
<td>11</td>
</tr>
<tr>
<td>GP-144</td>
<td>10</td>
</tr>
<tr>
<td>JBRGR-08-10-I</td>
<td>9</td>
</tr>
<tr>
<td>JBRGR-08</td>
<td>8</td>
</tr>
<tr>
<td>JBRGR-13-2</td>
<td>7</td>
</tr>
<tr>
<td>JBRGR-06-08</td>
<td>6</td>
</tr>
<tr>
<td>GBL-1</td>
<td>5</td>
</tr>
<tr>
<td>JBRGR-2-I</td>
<td>4</td>
</tr>
<tr>
<td>GBL-1</td>
<td>3</td>
</tr>
<tr>
<td>PLR-1</td>
<td>2</td>
</tr>
<tr>
<td>JBRGR-1</td>
<td>1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Band Number (Rm Value)</th>
<th>Esterase</th>
<th>Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>(0.05)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>(0.168)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>(0.305)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>(0.421)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>(0.068)</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>(0.171)</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>(0.252)</td>
<td>4</td>
<td>1</td>
</tr>
</tbody>
</table>

**Table 4.4**: Rm values of banding pattern of esterase and peroxidase at 12 days after germination.
The peroxidase isoenzymes were detected in all parts of the plant.

4.1.2.2. Peroxidase

4.1.2.3. Polyphenol oxidase (PPO)

was present in most of the genotypes except GP-181 and GP-184.

4.1.2.4. Table 4.2, having RM values of 0.068, 0.171 and 0.252. All 12 DAG three bands of peroxidase isoenzymes were observed.

Peroxidase

same way as in ettersase.

et al., 1993). The isoenzymatic profile of peroxidase was conducted as well as nonspecific Group known as (O-D) peroxidase (Abdel-Tawab specific enzymes such as NADP-peroxidase, fatty acid peroxidase etc.

and take part in defense mechanism. It includes many groups of predominately in leaves that increased when host-parasite interaction

The peroxidase isoenzymes present in all parts of plant.
Polymerotic in nature.

Eighteen genotypes except JGBR-1 and PLR-1, out of five bands three were 0.189. The ninth band of RM value 0.396 was present in all the sixteen genotypes except CP-1. Eight bands of RM value 0.270 among all the sixteen genotypes. The band of 0.189, CP-17, CP-175, CP-144, CP-1 and CP-181 showed the absence of Rz-znzyme. Bands of RM value 0.085 and 0.178 were present in all the isolates. Bands of RM value 0.085, 0.178, 0.399 and 0.493 were observed (Plate 4.3, Table 4.5) at 12 DAG for Acid Phosphatase.

Isoenzymatic pattern of ACP at 12 DAG


The mobilization of the phosphorous reserves during germination and the solubilization of macromolecular organic phosphate in soils during the solubilization of various plant sources plays an important role and phosphatases from various plants, fungi, animals and plants. The enzymes are ubiquitous in bacteria, fungi, animals and plants. The phosphatases are ubiquitous that releasing inorganic phosphate. These phosphatases is enzymes that hydrolyzes the terminal phosphate of the enzyme acid phosphatase is present in all plant cells. Acid

4.1.2.4 Acid Phosphatase

JGDR-13, L-2 and GBL-1. RM value 0.360 was present in most genotypes except PLR-1, GBR-1, L-2 and GBL-1. RM value 0.360, 0.090-0.175 and GBR-175. Band of 0.360, GBR-175, FRB-1, FRB-175, FRB-14, A band of RM value 0.130 was reported only in few except GP-14. A band of RM value 0.073 was present in all the sixteen genotypes of plant of RM value 0.073, 0.130, 0.192 and 0.260. All the bands were polymorphic in nature. The band of RM value 0.260, 0.073, 0.130, 0.192 and 0.360. All the bands were polymorphic in nature. The band of RM value 0.260, 0.073, 0.130, 0.192 and 0.360. All the bands were polymorphic in nature.
Plate 4.3: Polyacrylamide Gel Electrophoresis of Polyphenol Oxidase and Acid Phosphatase Isoenzymes at 12 days after germination.
<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Band Number (Rm Value)</th>
<th>Band Number (Rm Value)</th>
<th>Acid Phosphatase</th>
<th>Polyphenol Oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>GP-184</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-181</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-176</td>
<td>14</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-175</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-162</td>
<td>12</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-149</td>
<td>11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GP-144</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBCR-08-10-1</td>
<td>9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBCR-08-08</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBCR-13-2</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBCR-06-08</td>
<td>6</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB-1</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB-02-11</td>
<td>4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GB-1</td>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PR-1</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JBCR-1</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

 british sechelles at 12 days after germination.

Table 4.5: Rm values of banding pattern of polyphenol oxidase and acid phosphatase from the isozyme from...
ersase, respectively. In our study, only three and four bands were observed in peroxidase and
polymorphic enzymes was esterase followed by peroxidase. In our
bands in esterase with 261 accessions of *Capsicum*. The most
Battani et al. (2005) observed 15 bands in peroxidase and 37
were observed to be closely similar with 100% similarity.
found to be diverse one. Two genotypes namely GP-176 and JB-08-08
seven genotypes (GP-181, GP-144, JBER-08-10-1 and JB-08-08,
and GBL-1 showed nearly 95% similarity and remaining four
were clustered between clusters 'B2' and JBER-1) respectively. About 65% similarities
JB-08-08, GP-149, JBER-06-08, JBER-02-11, JBER-12, GP-176, GP-144,
(JB-08-08, JBER-06-08, GP-149, JBER-08-10-1, GP-144, GP-162,
and cluster B2 was further divided into two smaller clusters, 'B2a' and 'B2b',
including one (GP-175) and eleven genotypes (GP-181, GP-144,
and cluster BI was again divided into three sub-clusters and small clusters. Cluster II was separated
consisting of remaining fifteen genotypes which were again grouped
into two sub-clusters IIA and IIB. Sub-cluster IIA included only one
cluster II. Cluster I consisted of single solitary genotype (PLR-1'), Cluster II
reveals two main clusters I and II with the similarity range of 0.46 to
Jacobs's similarity coefficient. The dendrogram constructed (Fig.4.2),
Cluster analysis was carried out by the UPGMA method using
Cluster analysis of four isoenzymes:

- 0.38 between genotype GP-149 and JBER-06-08.
- Similarity ranged from 0.400 between genotype GP-184 and PLR-1 '

Genetic similarity of isoenzymes:
Figure 4.2: Dendrogram depicting the genetic relationships among 16 brinjal genotypes based on esterase, peroxidase, polyphenol oxidase, and acid phosphatase isoenzyme data.
observed between CP-1.49 and JBR-06-08.

In cluster B, the highest similarity value of 96% was
observed between CP-1.49 and JBR-06-08.

Cluster B included only one genotype (PL-1), and cluster B' consisted of
six genotypes (CP-1.49, JBR-02-11, CP-1.7, JBR-08-08 and
GEBR-01, GP-1.7, GP-1.8-08 and GEBR-08-08, respectively. Cluster B was split
to cluster B' and B'', with 57% similarity. Cluster B' consisted of
seven genotypes (GP-1.7, GP-1.8-08 and GEBR-01), respectively. Cluster B was split,
consisting of seven genotypes (GP-1.7, GP-1.8-08 and GEBR-01), respectively.

Cluster A, all including solitary

different clusters including seven and eight genotypes respectively.

The dendrogram constructed (Fig. 4.3, Table 4.7, showed two

The dendrogram constructed with all isoenzymes.

The study of isoenzymes revealed good relationships between

4.1.3 Pooled results of isoenzymes and protein:

due to narrow genetic base of cultivated genotypes selected in binhal.

Two main clusters were observed in our findings using four isozyymes
dehydrogenase, aspartate aminotransferase and malic enzyme. While
using five isozyymes viz., esterase, peroxidase, 6-phosphogluconate
phosphofructokinase constructed with 261 accessions of the genus Capsicum
Portera et al., (2005) also reported five groups based on the
Fig. 4.3: Dendrogram depicting the genetic relationship among 16 brinjal genotypes based on protein profiles and four isoenzymes.
4.2 DNA EXTRACtIONS

4.2 Molecular Markers (RAPD, ISSR, SSR)

(Alil and Javed, 2007)

Although DNA-based markers provide powerful tools for

binarial genotypes, a good tool for studying genetic diversity among

phenomenon explained that pooled study of isoenzymes alone with

pooled isoenzymes (0.46-0.100) was approximately similar in range

The average similarity among all the 16 binarial genotypes in

patterns of isoenzymes and protein via NATIVE-PAGE in binarial

protein polymorphism can be revealed by applied electrophoretic

tling to confirm that cultivar differences in

results of this research, one can confirm that cultivar differences in

and effective, especially in underdeveloped countries, based on the

evolutionary relationships, the capacity of protein markers is yet high

discerning variances within crop germplasm and for studying

cultivars (Alil and Javed, 2007)

remaining were checked and quantified on 0.8% agarose gel to confirm

samples measured through Nanodrop Spectrophotometer (Thermo

Tableau using Doyle and Doyle (1987) with little modification. The DNA

cultivated varieties, four SSVT lines and seven germplasm of Gujrat

DNA was extracted from sixteen genotypes comprising of five

samples were checked and quantified on 0.8% agarose gel to confirm

were of good quality and quantity. The yield of DNA isolated

from 199.16 ng/µl to 222.99 ng/µl with optical density near

about 1.80 indicating that DNA extracted was pure.
Further used in all amplification reactions, 10 μl of DNA was checked by loading 0.5 μl on 0.8% agarose gel and presence of 50 samples were made based on the total DNA yield. The diluted DNA RAPD, ISSR and SSR primers, Twenty-five μl dilution of the DNA samples were used for further marker analysis using

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Absorbance A260/A280 Ratio</th>
<th>Genotype</th>
<th>Str. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.071.20</td>
<td>1.80</td>
<td>GP-184</td>
<td>16.1</td>
</tr>
<tr>
<td>2013.98</td>
<td>1.87</td>
<td>GP-181</td>
<td>15.1</td>
</tr>
<tr>
<td>1028.60</td>
<td>1.68</td>
<td>GP-176</td>
<td>14.1</td>
</tr>
<tr>
<td>524.24</td>
<td>1.84</td>
<td>GP-175</td>
<td>13.1</td>
</tr>
<tr>
<td>423.19</td>
<td>1.84</td>
<td>GP-172</td>
<td>12.1</td>
</tr>
<tr>
<td>5280.76</td>
<td>1.85</td>
<td>GP-149</td>
<td>11.1</td>
</tr>
<tr>
<td>1107.42</td>
<td>1.92</td>
<td>GP-144</td>
<td>10.1</td>
</tr>
<tr>
<td>669.99</td>
<td>1.88</td>
<td>GP-08.10.1</td>
<td>9.1</td>
</tr>
<tr>
<td>2225.99</td>
<td>1.93</td>
<td>GP-08.08</td>
<td>8.1</td>
</tr>
<tr>
<td>503.06</td>
<td>1.84</td>
<td>GP-13.2</td>
<td>7.1</td>
</tr>
<tr>
<td>203.65</td>
<td>1.78</td>
<td>GP-06.08</td>
<td>6.1</td>
</tr>
<tr>
<td>911.22</td>
<td>1.79</td>
<td>GB-01</td>
<td>5.1</td>
</tr>
<tr>
<td>830.28</td>
<td>1.93</td>
<td>GB-02-11</td>
<td>4.1</td>
</tr>
<tr>
<td>1361.95</td>
<td>1.81</td>
<td>GBL-1</td>
<td>3.1</td>
</tr>
<tr>
<td>199.16</td>
<td>1.78</td>
<td>PLR-1</td>
<td>2.1</td>
</tr>
<tr>
<td>2083.16</td>
<td>1.74</td>
<td>JBER-1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 4.8 Purity and Concentration of DNA samples in brinjal
4.2.2 RAPD

The RAPD was used for characterization of brinjal genotypes for studying genetic diversity and relationships among them. In present investigation, 16 brinjal genotypes were subjected to RAPD analysis using 12 different random primers of Operon series such as OPC, OPW, OPA, OPL, and OPB.

Initially, 12 RAPD primers were screened using genomic DNA of JBRG-1 genotype. Out of 12 RAPD primers used, only seven primers; OPA-01, OPW-11, OPC-05, OPB-09, OPB-12 and OPL-15, OPC-07, OPC-11 and OPC-14 didn't amplified the product. Therefore, seven primers were utilized for further diversity analysis. The reproducibility of the appearance of RAPD banding patterns was confirmed by at least two times of repetition analyses. (Plate 4.4 to 4.6)

4.2.2.1 Polymorphism pattern of RAPD

Seven primers amplified a total of 50 bands/alleles out of which 47 bands were polymorphic with an average of 7.14 bands per primer, while remaining three bands were monomorphic (Plate 4.4 to 4.6 and Table 4.9). Five primers gave 100% polymorphism, while two primers viz., OPC-05 and OPB-01 showed monomorphic bands. The average polymorphism was 95.01%. The size of the amplified fragments ranged from 216.97 to 2919.89 bp. The largest (2919.89 bp) and the smallest (216.97 bp) fragment were amplified by OPB-01 and OPC-09 primer, respectively.
Plate 4.4: RAPD markers of brinjal genotypes generated by

OPA-01, OPM-11 and OPC-05.
Plate 4.5: RAPD markers of bininal genotypes generated by OPB-01 and OPA-09.
<table>
<thead>
<tr>
<th>RPI</th>
<th>PIC×A</th>
<th>PIC Value</th>
<th>(P/A)</th>
<th>Polymerorphism</th>
<th>bands</th>
<th>No. of Allele/Band size</th>
<th>RAPD</th>
<th>Primers</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.882</td>
<td>0.807</td>
<td>95.01%</td>
<td>50</td>
<td>3</td>
<td>4</td>
<td>43</td>
<td>Total</td>
<td></td>
</tr>
<tr>
<td>8.540</td>
<td>0.854</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>9</td>
<td>216.97-259.22</td>
<td></td>
</tr>
<tr>
<td>6.840</td>
<td>0.855</td>
<td>100</td>
<td>8</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>417.20-259.28</td>
<td></td>
</tr>
<tr>
<td>4.728</td>
<td>0.788</td>
<td>100</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>7</td>
<td>362.18-1585.25</td>
<td></td>
</tr>
<tr>
<td>9.735</td>
<td>0.885</td>
<td>81.8</td>
<td>2</td>
<td>0</td>
<td>9</td>
<td>274.41-2919.89</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.434</td>
<td>0.739</td>
<td>83.3</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>4</td>
<td>246.36-1070.10</td>
<td></td>
</tr>
<tr>
<td>3.000</td>
<td>0.750</td>
<td>100</td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>480.26-1182.67</td>
<td></td>
</tr>
<tr>
<td>3.895</td>
<td>0.779</td>
<td>100</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td>1</td>
<td>260.82-1085.43</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.9: Size, number of amplified bands, percent polymorphism, PIC and RPI obtained by RAPD.
to 0.885 with an average of 0.807 per primer. Which was low as compared to present PIC value ranging from 0.739 values ranged from 0.100 to 0.499 with an average PIC score of 0.310 ranged from 3 to 18 with an average of 8.3 bands per primer. PIC (27.5%) were polymorphic. The number of bands produced per primer primes on 19 advanced cultivars and landraces of brinjal, of which 66 primers (OP-B-01) obtained 240 amplicons by using 29 RAPD

Tiwari et al. (2009) obtained 240 amplicons by using 29 RAPD

substantially higher for OP-B-01 primer than other primers (OP-B-01) with an average of 5.882. The primer index value was RAPD polymorphic Index varied from 3.000 (OP-W-11) to 9.735

monomorphic bands. Calculating shared polymorphic, shared unique and total number of

RAPD bands, the seven RAPD primers was depicted in the form of graph (Fig. 4.5) by

properties of polymorphic and monomorphic bands amplified by

OP-C-05, OP-C-07, OP-C-02, OP-C-09, OP-C-12 and OP-C-09. The polymorphic information content (PIC) was calculated for each primer (Table 4.8) and it was varied between 0.739 one unique bands was observed in four primers (OP-C-05, OP-C-07, OP-W-11.

Lowest number of bands (4) was obtained in case of OP-W-11.

Findings.

primer (OP-B-01) on ten varieties of brinjal, which support present

also reported the maximum number of 14 bands by using same

OP-B-01 primer followed by 10 bands (OP-C-09). Biswas et al (2009) The maximum numbers of 11 bands were produced by
Cluster B1 was split into two sub-clusters B1a and B1b, including three and
Cluster B2 consisted of only one genotype GP-176 and cluster B2 was
split into two sub-clusters B2a and B2b. Cluster B2 included only the divergent genotypes (GP-181 and
similarity. Cluster II was divided into two sub-clusters; sub-cluster A
diverse genotypes (PLR-1) out-grouped to other 15 genotypes with 82%
diverse genotypes in Cluster II. The cluster-I consisted of only 16
in Fig. 4.8. Sixteen binatal genotypes were grouped into two main
similarity coefficient for RAPD data of 16 binatal genotypes is depicted
The dendrogram constructed using UPGMA based on Jaccard’s

using NTYSUP 2.02I software (Table 4.10 and Fig. 4.6).

**4.2.3 Cluster analysis of RAPD**

Present findings:

Similitude values ranging from 0.69 to 0.891 which support the
selected by Tiwari et al. (2009). Similtude ranged from 0.69 to 0.891 which support the
investigation, result similarity coefficient ranged from 0.891 to 0.963 with an average similarity of 0.899, while in present
values between 19 parents of eggplant cultivars ranging from 0.84 to
Tiwari et al. (2009) observed Jaccard’s similarity coefficient
indicating that these two genotypes must have less genetic diversity.
coefficients (0.69) was found between JGBP-09-08 and JBR-02-11
observed between GP-181 and PLR I and the highest similarity
similarity ranged from 0.188 to 0.969. Lowest similarity (0.188) was
according to Jaccard’s similarity coefficient (Table 4.10). Genetic

4.2.2 Genetic Similarity among varieties based on RAPDs:
Table 4.10: Jaccard’s similarity coefficient of 16 binomial genotypes based on RAPD data.
Figure 4.6: Dendrogram depicting the genetic relationship among 16 bacterial genotypes based on the RAPD data.
main clusters were observed at a similarity value of 0.280.

accessions at a similarity value of 0.757. While in present analysis two major groups of pepper
Sithiwong et al., (2005) observed two major groups of pepper
matrix of data and dendrogram was in the best scale.

findings correlation coefficient was 0.869 indicating that similarity
matrix of data and dendrogram was in the poor scale. While in present
indicated that the correlation coefficient between the similarity matrix
distance and Euclidean distance (r = -0.17) and negative correlation
The Mantel test showed that there is no correlation between genetic
population of Solanum species based on UPGMA analysis of the data.

Stedje et al., (2003) revealed no clear groupings of the 18

"Good ate."

categorized by Rohlf (1994) was found to fall under the category of

goodness of fit for a cluster analysis (matrix correlation r = 0.869)
as
investigation the mean test statistic Z was normalized and degree of
the present study were shown in Fig. 4.7. As the phenotypic correlation coefficient
the plot and statistics of 16 principal genotypes included in present
the association statistics were made and calculated by MAXCOMP.
The principal genotypes were compared to the original matrices
of phenotypic values were also computed using the program COPH.
To test the goodness of fit of the clustering of RAPD data, matrix

investigation.

eggplant varieties into two main clusters similar as present
JB-RO-20, 11, Birois et al. (2009) observed segregation of the ten
similarities (91%) was observed between the genotypes JBGR-06-08 and
JB-RO-20, 11, GBR-1 and JB-RO-08-08. Among cluster B2i, highest
GBL-1, JBGR-08-10, GP-144, GP-149, JBGR-06-08,
similarities. The ten genotypes included in cluster B2i were JBGR-1,
92% and GP-184 formed clusters B2i. are nearly shared 98%
ten genotypes, respectively. The three genotypes namely GP-175, GP-

Figure 4.7: Cophenetic values against Jaccard's similarity coefficients from RAPD data of 16 bacterial strains.

Matrix correlation: r = 0.869

(normalized Mantel test statistic Z)
substantially higher in UBC-823 than other primers. For ten primers, ISSR primer index values was 9.29 with an average 5.26 per primer. ISSR polymorphism index varied from 0.46 to 0.72. The average value of PIC was given by two primers UBC-810 and UBC-823. The largest PIC value i.e. 0.845 was each primer which was ranged from 0.231 to 0.845. Smallest PIC value was given by UBC-813 and the largest PIC value by UBC-823 (11). The PIC was calculated for UBC-823 and least by UBC-813 (2). The PIC was calculated for each primer which were obtained by primer fragment were amplified by UBC-900 and UBC-810 primer; 2951.62 bp. The largest (2951.62 bp) and the smallest (196.35 bp) were 4.11. The size of the amplified fragments ranged from 196.35 to 100% polymorphism with an average 6.8 bands per primer. A total of 68 bands were obtained from the ten ISSR primers and all showed was confirmed by at least two times of repetition analyses. A total of

The reproducibility of the appearance of ISSR banding patterns

4.2.3.1 Polymorphism Pattern of ISSR

(Plate 4.17 to 4.10)

Screening was carried out using ten primers of ISSR of UBC
UBC-807, UBC-810 and UBC-813.

Plate 4.7: ISSR markers of birchland genotypes generated by

UBC-813

UBC-810

UBC-807
Plate 4.9: ISSR markers of bininal genotypes generated by
UBC-822, UBC-823, and UBC-824.
PIC = Polymorphism Information Content; IPI = ISSR Primer Index

<table>
<thead>
<tr>
<th>IPI</th>
<th>% Polymorphism</th>
<th>(A)</th>
<th>Total No. of Allele</th>
<th>Mono-allelic Bands</th>
<th>(B)</th>
<th>Total No. of Polymeric Bands</th>
<th>(B/p)</th>
<th>Allele/Band size</th>
<th>ISSR Primers</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.18</td>
<td>0.818</td>
<td>100</td>
<td>0</td>
<td>6</td>
<td>3</td>
<td>2</td>
<td>18</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>4.84</td>
<td>0.807</td>
<td>83.3</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td>6</td>
</tr>
<tr>
<td>6.37</td>
<td>0.797</td>
<td>100</td>
<td>11</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td>11</td>
</tr>
<tr>
<td>9.29</td>
<td>0.845</td>
<td>100</td>
<td>9</td>
<td>9</td>
<td>1</td>
<td>9</td>
<td>1</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>4.65</td>
<td>0.742</td>
<td>100</td>
<td>12</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>9.15</td>
<td>0.720</td>
<td>100</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td>9</td>
</tr>
<tr>
<td>5.94</td>
<td>0.799</td>
<td>100</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>1</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>5.00</td>
<td>0.231</td>
<td>100</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>0</td>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>6.76</td>
<td>0.845</td>
<td>100</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>2.86</td>
<td>0.670</td>
<td>100</td>
<td>4</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td></td>
<td></td>
<td>4</td>
</tr>
</tbody>
</table>

Table 4.1: Size, number of amplified bands, percent polymorphism, PIC and IPI obtained by ISSR Primers.
Pic was found to be 0.88.

from 27 to 85, with fragment size ranging from 192 to 2629 bp. Mean
primers, all of which were polymorphic. The number of bands varied
bands from 13 genotypes of Capsicum using three selected ISSR
Peral et al. (2011) observed total of 204 clear and reproducible

Present results. Primers except one produced 100% polymorphic bands which support
primers. The amplified product ranged from 200 to 1,600 bp. All
primers varied from 10 to 21, with an average of 16.8 fragments per
Mexican Hush tomato species and found out the number of bands per
Vargas-Ponce et al. (2010) used six ISSR primers on eight
6.8 bands per primer.

While present study showed 2 to 11 bands per primer with an average
primers ranged from 6 to 21 with an average of 13 bands per primer.
and four cultivars and generated 299 amplifications from 23 primers, of which
Tiwari et al. (2009) evaluated ISSR assay of 19 binodal cultivars

binal genotypes.

polymorphisms was observed with nine ISSR primers tested on sixteen
polymorphisms was 99.1%, while in present study 100%
obtained from 54 of the 100 primers tested, and the percentage of
Solanum species. A total of 552 polymorphic amplified bands were
eggplant (Solanum melongena) and 12 accessions in eight related

Ishihara et al. (2008) performed ISSR analysis in eight cultivars of
monomorphic bands.

calculating shared polymorphic, shared unique and total number of
the ten ISSR primers was depicted in the form of graph (Fig. 4.8).

Properties of polymorphic and monomorphic bands amplified by
was found between JBP-08-08 and JBP-08-10.1, while highest similarity 0.724 was observed between CP-149 and PLR-1. According to Jaccard’s similarity coefficient, lowest similarity 0.170 calculated using Jaccard’s similarity coefficient given in Table 4.12. Genetic similarity between varieties based on ISSR was amplified by the ISSR primers.

Fig. 4.8: Profiles of polymorphic and monomorphic bands.
| GP-1.84 | GP-1.81 | GP-1.76 | GP-1.75 | GP-1.62 | GP-1.49 | GP-1.44 | GP-1.41 | GP-1.38 | GP-1.35 | GP-1.32 | GP-1.29 | GP-1.25 | GP-1.21 | GP-1.18 | GP-1.15 | GP-1.12 | GP-1.09 | GP-1.06 | GP-1.03 | GP-1.00 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0.579  | 0.397  | 0.478  | 0.558  | 0.638  | 0.718  | 0.798  | 0.878  | 0.958  | 1.038  | 1.118  | 1.198  | 1.278  | 1.358  | 1.438  | 1.518  | 1.598  | 1.678  | 1.758  | 1.838  | 1.918  |
| 0.576  | 0.395  | 0.476  | 0.556  | 0.636  | 0.716  | 0.796  | 0.876  | 0.956  | 1.036  | 1.116  | 1.196  | 1.276  | 1.356  | 1.436  | 1.516  | 1.596  | 1.676  | 1.756  | 1.836  | 1.916  |
| 0.574  | 0.394  | 0.474  | 0.554  | 0.634  | 0.714  | 0.794  | 0.874  | 0.954  | 1.034  | 1.114  | 1.194  | 1.274  | 1.354  | 1.434  | 1.514  | 1.594  | 1.674  | 1.754  | 1.834  | 1.914  |
| 0.572  | 0.393  | 0.473  | 0.553  | 0.633  | 0.713  | 0.793  | 0.873  | 0.953  | 1.033  | 1.113  | 1.193  | 1.273  | 1.353  | 1.433  | 1.513  | 1.593  | 1.673  | 1.753  | 1.833  | 1.913  |
| 0.570  | 0.392  | 0.472  | 0.552  | 0.632  | 0.712  | 0.792  | 0.872  | 0.952  | 1.032  | 1.112  | 1.192  | 1.272  | 1.352  | 1.432  | 1.512  | 1.592  | 1.672  | 1.752  | 1.832  | 1.912  |

Table 4.12: Jaccard's similarity coefficient of 16 botanical genotypes based on ISSR data.
The sub-cluster II consisted of 10 genotypes viz. GP-08-10-1, JBP-08-10-1, and JBP-08-08. The highest similarity was observed between variety JBP-08-08 and JBP-08-10-1 having approximately 60% similarity. Cluster II and cluster III were further subdivided into cultivar- and variety-specific clusters. Cluster II included three genotypes viz. GBL-1, GBR-08-08 and JBP-08-10-1. Cluster II was divided into two clusters IIb and IIa. The sub-cluster IIa consisted of 10 genotypes (GP-149) in the main cluster I and II were 29% similar. The highest similarity was observed with only one genotype (GP-149) in the main cluster I. The presence of ISSR markers clearly resolved Solanum genotypes into two main clusters. The dendrogram obtained with ISSR markers is given in (Fig. 4.9). The dendrogram obtained with ISSR markers, ranging from 0.03 to 0.33, coefficient values using Jaccard's coefficient based on five ISSR coefficients ranged from 0.17 to 0.22 indicating that genotypes selected were more diverse. The values were also correlated using the Jaccard's similarity coefficient between study participants.
ISSR analysis would be useful also for genetic mapping of crape
juniors. Since the present ISSR markers were highly polymorphic,
evidence of high applicability of this analysis and gave some new
among 16 eggplant genotypes. The present data provided enough
marker was used as tools for assessing phylogenetic relationships
seven clusters by using 52 ISSR markers. In present study, the ISSR
(Stahl et al. 2008) clearly distinguished eight Solanum species into
capsicum based on five ISSR markers which support present findings.
Patel et al. (2011) and two major clusters of 13 genotypes of

touted."

categorized by Rohlf (1994) was found to fall under the category of
analysis was performed to determine the degree of
also the Mantel test statistics Z was normalized and degree of
program COPH in same way as in RAPD analysis. In the present study
(Fig. 4.10), matrix of cophenetic values were also computed using the
to test the goodness of fit of clustering of ISSR data


Similar, gp-184 and JBR-02-11 showed 59% similarity with variety
JBR-02-12, GP-176, gp-173, JBR-13-2 and JBGFR-06-08 were nearly 70%
Fig. 4.10: Cophenetic values against Jaccard's similarity coefficients from ISSR data of 16 brinjal genotypes.

Matrix correlation: $r = 0.834$ (normalized Mantel test statistic $Z$)
Plate 4.11: SSR markers of binual genotypes generated by smMSSR09, smMSSR11 and smMSSR12.
Plate 4.12: SSR markers of bininal genotypes generated by smSSR14 and smSSR15.
<table>
<thead>
<tr>
<th>PIC (b/a)</th>
<th>PIC Value</th>
<th>Polymorphism (b/a) %</th>
<th>Total No. of Monomorphic bands</th>
<th>No. of Monomorphic bands</th>
<th>No. of Bands (b)</th>
<th>No. of Allele/Genotype size</th>
<th>PRIMERS (SSR)</th>
<th>PRIMERS (S)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.92</td>
<td>0.534</td>
<td>100</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>1.45</td>
<td>0.485</td>
<td>100</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>1.21</td>
<td>0.405</td>
<td>100</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>2.87</td>
<td>0.718</td>
<td>50</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
<td>100</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>2.68</td>
<td>0.671</td>
<td>100</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>2.74</td>
<td>0.686</td>
<td>100</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>0.320</td>
<td>0.561</td>
<td>50</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>0.143</td>
<td>0.715</td>
<td>100</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
<tr>
<td>3.89</td>
<td>0.718</td>
<td>50</td>
<td>32</td>
<td>5</td>
<td>4</td>
<td>21</td>
<td>112.10-325.66</td>
<td>113.25-575.22</td>
<td>71.80-224.639</td>
</tr>
</tbody>
</table>

**Notes:**
- Polymorphism = PIC = SSR Primer Index
- Shared, n = Unique
- **Table 4.13:** Size, number of amplified bands, per cent polymorphism, PIC and SPI obtained by SSR

---

**Legend:**
- PIC = Polymorphism Information Content
- SPI = SSR Primer Index
- S = Shared
- n = Unique
0.35 to 0.85. However, in the present study, genetic similarity ranged from 0.45 to 0.98. However, in the present study, genetic similarity ranged from

The sixty tomato entries included were 0.71 with the values ranging from

Benet et al., (2008) found that average genetic similarity among

was found between GOB-1 and JBR-02-11. Observed between CP-149 and JBR-1, while the highest similarity (0.857) similarity ranged from 0.385 to 0.857. Lowest similarity 0.385 was according to Jacaré's similarity coefficient (Table 4.14). Genetic

4.2.4.2 Genetic Similarity among Varieties based on SSRs:

From 2 to 7 with an average of 4.5. The number of alleles ranged

primers amplified fragments (alleles), the number of alleles ranged

70 entries by means of SSR analysis at 21 loci. Seventeen out of 23

Sumner et al. (2010) estimated the pattern of genetic variation among

more frequently of polymorphism was observed in the present study.

polymorphism among Egyptian cultivars and lines was lower while

were monomorphic. These data suggested that the frequency of

polymorphism, monomorphic species, with an average of 17.7% of local producing PCR products

Squillace et al. (2003) have reported that, of the 25 varieties of

were found to be five out of 32 bands. An average of 3.2 fragments per primer. Numbers of monomorphic bands

were observed with amplified the PCR product and 86% polymorphism was observed with

1054 primer pairs, in present study. Out of 12 primers 10 primers

monomorphic, resulting in an average of 2.2 alleles per locus over

polymorphism among eight lines of S. melongena, while 46 were

were monomorphic bands. Numbers of polymorphic and monomorphic bands amplified by

calculated shared polymorphic, shared unique and total number of

the ten SSR primers was depicted in the form of graph (Fig 4.11) By

Properties of polymorphic and monomorphic bands amplified by
4.2.3 Cluster analysis of SSR

Figure 4.11: Properties of polymorphic and monomorphic bands

- Shared Polymorphic
- Monomorphic
- Shared Unique
- Unique

The dendrogram in Figure 4.12 was used to draw the dendrogram of each plant species. The derived trees and plots are shown in Figure 4.12.

The analyzed data were then used to draw a dendrogram. In this study, grouped based on their presence and absence as 1 and 0. These grouped for the analysis of SSR data, the results of the experiments were amplified by the SSR primers.
Table 4.14: Jaccard’s similarity coefficient of 16 brinjal genotypes based on SSR data.

<table>
<thead>
<tr>
<th>GP-184</th>
<th>GP-181</th>
<th>GP-176</th>
<th>GP-175</th>
<th>GP-144</th>
<th>GP-149</th>
<th>JBG-GR-OB-08-08</th>
<th>JBG-GR-13.2</th>
<th>JBG-GR-06-08</th>
<th>GOB-1</th>
<th>JBG-GR-02.11</th>
<th>GBL-1</th>
<th>PIR-1</th>
<th>JBG-IR</th>
<th>GP-02.11</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.476</td>
<td>0.455</td>
<td>0.652</td>
<td>0.619</td>
<td>0.636</td>
<td>0.695</td>
<td>0.588</td>
<td>0.650</td>
<td>0.545</td>
<td>0.591</td>
<td>0.591</td>
<td>0.667</td>
<td>0.662</td>
<td>0.619</td>
<td>1.000</td>
</tr>
<tr>
<td>0.619</td>
<td>0.842</td>
<td>0.478</td>
<td>0.669</td>
<td>0.700</td>
<td>0.721</td>
<td>0.678</td>
<td>0.571</td>
<td>0.619</td>
<td>0.619</td>
<td>0.609</td>
<td>0.619</td>
<td>0.652</td>
<td>0.591</td>
<td>0.857</td>
</tr>
<tr>
<td>0.478</td>
<td>0.478</td>
<td>0.669</td>
<td>0.500</td>
<td>0.550</td>
<td>0.565</td>
<td>0.700</td>
<td>0.550</td>
<td>0.621</td>
<td>0.695</td>
<td>0.783</td>
<td>0.783</td>
<td>0.619</td>
<td>0.609</td>
<td>1.000</td>
</tr>
<tr>
<td>0.478</td>
<td>0.478</td>
<td>0.669</td>
<td>0.500</td>
<td>0.550</td>
<td>0.565</td>
<td>0.700</td>
<td>0.550</td>
<td>0.621</td>
<td>0.695</td>
<td>0.783</td>
<td>0.783</td>
<td>0.619</td>
<td>0.609</td>
<td>1.000</td>
</tr>
<tr>
<td>0.669</td>
<td>0.669</td>
<td>0.700</td>
<td>0.721</td>
<td>0.678</td>
<td>0.571</td>
<td>0.678</td>
<td>0.571</td>
<td>0.619</td>
<td>0.695</td>
<td>0.783</td>
<td>0.783</td>
<td>0.619</td>
<td>0.609</td>
<td>1.000</td>
</tr>
<tr>
<td>0.669</td>
<td>0.669</td>
<td>0.700</td>
<td>0.721</td>
<td>0.678</td>
<td>0.571</td>
<td>0.678</td>
<td>0.571</td>
<td>0.619</td>
<td>0.695</td>
<td>0.783</td>
<td>0.783</td>
<td>0.619</td>
<td>0.609</td>
<td>1.000</td>
</tr>
<tr>
<td>0.695</td>
<td>0.695</td>
<td>0.721</td>
<td>0.678</td>
<td>0.571</td>
<td>0.678</td>
<td>0.571</td>
<td>0.678</td>
<td>0.619</td>
<td>0.695</td>
<td>0.783</td>
<td>0.783</td>
<td>0.619</td>
<td>0.609</td>
<td>1.000</td>
</tr>
<tr>
<td>0.588</td>
<td>0.588</td>
<td>0.650</td>
<td>0.545</td>
<td>0.591</td>
<td>0.591</td>
<td>0.662</td>
<td>0.619</td>
<td>0.609</td>
<td>0.619</td>
<td>0.857</td>
<td>0.662</td>
<td>0.619</td>
<td>0.591</td>
<td>1.000</td>
</tr>
<tr>
<td>0.545</td>
<td>0.545</td>
<td>0.591</td>
<td>0.591</td>
<td>0.667</td>
<td>0.619</td>
<td>0.619</td>
<td>0.609</td>
<td>0.619</td>
<td>0.857</td>
<td>0.662</td>
<td>0.619</td>
<td>0.591</td>
<td>0.857</td>
<td>1.000</td>
</tr>
<tr>
<td>0.591</td>
<td>0.591</td>
<td>0.667</td>
<td>0.619</td>
<td>0.619</td>
<td>0.609</td>
<td>0.857</td>
<td>0.662</td>
<td>0.619</td>
<td>0.619</td>
<td>0.662</td>
<td>0.619</td>
<td>0.591</td>
<td>0.857</td>
<td>1.000</td>
</tr>
<tr>
<td>0.667</td>
<td>0.667</td>
<td>0.619</td>
<td>0.609</td>
<td>0.619</td>
<td>0.609</td>
<td>0.619</td>
<td>0.609</td>
<td>0.857</td>
<td>0.619</td>
<td>0.662</td>
<td>0.619</td>
<td>0.591</td>
<td>0.857</td>
<td>1.000</td>
</tr>
<tr>
<td>0.571</td>
<td>0.571</td>
<td>0.619</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.619</td>
<td>0.857</td>
<td>0.619</td>
<td>0.619</td>
<td>0.591</td>
<td>0.857</td>
<td>1.000</td>
</tr>
<tr>
<td>0.662</td>
<td>0.662</td>
<td>0.619</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.619</td>
<td>0.857</td>
<td>0.619</td>
<td>0.619</td>
<td>0.591</td>
<td>0.857</td>
<td>1.000</td>
</tr>
<tr>
<td>0.619</td>
<td>0.619</td>
<td>0.619</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.857</td>
<td>0.619</td>
<td>0.619</td>
<td>0.619</td>
<td>0.591</td>
<td>0.857</td>
<td>1.000</td>
</tr>
<tr>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.857</td>
<td>0.619</td>
<td>0.619</td>
<td>0.619</td>
<td>0.591</td>
<td>0.857</td>
<td>1.000</td>
</tr>
<tr>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.609</td>
<td>0.857</td>
<td>0.619</td>
<td>0.619</td>
<td>0.619</td>
<td>0.591</td>
<td>0.857</td>
<td>1.000</td>
</tr>
<tr>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
<td>0.857</td>
</tr>
</tbody>
</table>
SSR data.

PIE 4.12: Dendrogram depicting the genetic relationship among 16 birch genotypes based on the SSR analysis.
Clustered distinctly were from different geographical locations. Subgroups were mostly from the same origin and those, which
similar genetic background. Those clustered within the same group or
according to their geographic location, and thus, might have a
revealed that the genotypes were grouped in different clusters.

The dendrogram developed by SSR based primer analysis

To fall under the category of "amyloid"

Matrix correlation R = 0.72 (as categorized by Rohlf (1994) was found
normalized and also the degree of goodness of fit for a cluster analysis
4:13). In the present study also, the mantle test statistics Z
comparative values were also computed using the program COPHY.

To test the goodness of fit of the clustering of SSR data, matrix of

Variety GP-118 and PR-L1 also showed 82% similarity.

Similarity about 86% was observed between JBR-02-11 and GP-118,
GP-176, GP-117, GP-177, JBR-02-11, GP-118, and PR-L1. The highest
of the genotypes namely of GP-117, GP-115, GP-112, JBR-08-08, JBR-08-08,
GP-144 showed similarity of nearly 78%. The sub-cluster “A” comprised
GP-144, GP-08-10-1, JBR-08-08-10-1 and GP-144, among them JBR-08-08-10-1
and GP-144 (GBR-1), the sub-cluster “B” consisted of three genotypes namely
of two clusters I and II. The sub-cluster B2 consists of one genotype
sub-clusters B1 and B2. The sub-cluster B1 is again subdivided into
two and shared at similarity about 64%. Cluster B was split into two
cluster A consisted of two genotypes namely JBR-13-2 and GP-184
second cluster consisted of two sub-clusters A and B. The first sub-
classic I was solitary and consisted of only one genotype (GP-149). The

Table 4:14). The dendrogram based on SSR data, precisely organized
coefficient and UPGMA using NTSYSpc-2.02i software respectively.

Similarity index and cluster analysis was done by jaccard's
Figure 4.13: Cophenetic values against Jaccard's similarity coefficients from SSR data from 16 brachiaria genotypes.

Matrix correlation: $r = 0.72$
varieties that showed low similarity are diverse and of different origin.

Popular cultivar and SSVT are of Junagadh, Gujrat and the two and PLR-1. The two varieties that showed high similarity were the and JBP-02-11 and lowest genetic similarity found between GP-149 and JBP-02-08. The highest genetic similarity was observed between varieties JBP-02-08 4.15. The mean genetic similarity among the varieties was 0.521. The the pooled data set comprising of RAPD, ISSR and SSR markers (Table 4). The genetic similarity values ranged from 0.281 to 0.761 with

4.2.5 Genetic similarity among varieties based on pooled data:

Similarity between 16 binuclear genotypes molecular markers which can be used to confirm the difference and molecular study was conducted using all (Khan et al., 2006). Thus, the pooled study was conducted using all particular marker does not fulfill the goal of identification of genotype one can differentiate genotypes from one another. The reliability of one based on molecular marker study through RAPD, ISSR, SSR.

4.2.6 Pooled study of molecular markers:

observed in present study. similarity value of 0.84. However, the poor hit value of "0.72" was groups were distinguished by truncating the dendrogram at genetic which have quite a good fit to the genetic similarity matrix. Four binural using the 35 polymorphic loci resulted in the dendrogram, pattern following UPGMA cluster analysis of the 39 improved lines of present findings. Bener et al. (2008) studied the genetic diversity clusters (Sunsent et al., 2010). Thus result was in agreement with 23 SSR primers showed two large main clusters and several small. The dendrogram obtained from 70 scatter explain entities using
Category of "good fit".

and degree of goodness of fit for a cluster analysis (matrix correlation r) was found to fall under

In the present study also, the Mantel test statistics were normalized and computed using the program COPH in the same way as in RAPD analysis. Marker data (F1; 4; 15), matrix of co-phenetic values were also computed.

To test the goodness of fit of clustering of pooled molecular whole data set, when compared with each other as well as when compared with the SSRs showed a high degree of similarity in dendrogram topologies of the present study, the two marker types namely ISSR and similarity 0.76.

Genotypes namely JBR-02-11 and JBR-06-08 showed the highest together with cluster 2. Cluster 1 consisted of two genotypes viz. GP-175 and GP-176. Cluster 2 was further split into two clusters, 2.I. 2.2. and 2.II. including three genotypes, respectively, cluster 2.I included three genotypes, whereas cluster 2.II. included only one genotype PPR-1 and sub-cluster B was again subdivided into two sub-cluster A and B. Sub-programme 1 consisted of single genotype PPR-14, indicating that it was more diverse and could be used as one of the parents in the breeding programme. Cluster II was divided into two sub-clusters A and B. Sub-clusters I and II were clearly resolved at 39% similarity value. main clusters I and II were clearly resolved at 39% similarity value.

The pairwise genetic distance based on the pooled data set was 4.25.2 Cluster Ring Pattern based on pooled molecular data.
Data of RAPD, ISSR and SSR

Figure 4.14: Dendrogram depicting the genetic relationship among 16 binomial genotypes based on pooled data.
Fig. 4.15: Cophenetic values against Jacard’s similarity coefficients from pooled data of RAPD, ISSR

Matrix correlation: r = 0.83

normalized Mantel test statistic (Z)
Similar result was obtained in case of present findings. Compared the same accession regardless of the marker type used, and SSR grouped most of the accessions into two main clusters, which by SSR and RAPD. The clustering pattern obtained with RAPD, AFLP, and the highest in RAPD in 30 Solanum accessions, while in SSR and the highest in ISSR, followed by ISSR. S. et al. (2009) revealed the lowest similarity values through pattern of cluster separation was found in present findings. Some analysis of pooled data of three molecular markers. Some main clusters and several small clusters. In present study we found 1 = 0.83 by analysis of pooled data of three molecular markers. Same scatter graph, genotypes, based on significant correlation to estimate the genetic distances among 70 diversity existed among the selected sixteen binarial genotypes. study for all three marker systems confirmed the high phenotypic (4.37%). The high level of polymorphism observed in the present (9.5%) followed by RAPD (94%), and the microsatellite analyses were 27. The highest level of polymorphism was obtained by ISSR were 69 and by SSR fragments amplified by RAPD was 47, by ISSR were 69 and by SSR fragments amplified by RAPD was 50, 10 ISSR markers.

4.2.5.3 Comparison of RAPD, ISSR and SSR markers:
Genotypes.

Biochemical and molecular data clearly differentiated the sixteen similarity value (77%) the clustering pattern depicted with pooled genotypes, namely JBR-02-I, JBR-09-00-8, and JBR-11-I, among which two
1, JBR-02-08, JBR-02-11, JBR-09-00-8, and JBR-11-I, who composed of two
other genotypes (GP-14, JBR-09-08, 1, and JBR-11-I). The similarity of the
1, JBR-02-08, JBR-02-11, JBR-09-00-8, and JBR-11-I, who were separated into two small clusters C and C. However, it consists of
cluster IIIB at the similarity range of 55%. The cluster IIIB was again
respectively, the two genotypes namely GP-14, and GP-176 and GP-181 formed
into small cluster IIIB and IIIB with two and two larger genotypes.
1, JBR-02-08, and JBR-11-I, which were again subdivided
IIIB at 47% similarity range. The sub-cluster IIIB included only
genotypes. The main cluster II was partitioned into two sub-clusters
replacing that this genotype was more diverse among all the
cluster I and II, Genotype P1R-I was present single in cluster I
(Table 1). The dendrogram constructed formed two distinct
similar with the pooled study of molecular markers and isozymes
UPGMA method differentiated genotypes and results obtained were
The Jackard's similarity coefficient and cluster analysis with the
biochemical markers alone.

Genotypes in a better way than either with only molecular or with
analyzed collectively to find out whether it could differentiate

The data of all molecular and biochemical markers were

4.3 Pooled Study of all biochemical and molecular markers:
<table>
<thead>
<tr>
<th>GP</th>
<th>1.000</th>
<th>0.417</th>
<th>0.433</th>
<th>0.558</th>
<th>0.534</th>
<th>0.554</th>
<th>0.448</th>
<th>0.510</th>
<th>0.431</th>
<th>0.452</th>
<th>0.432</th>
<th>0.335</th>
<th>0.322</th>
<th>0.358</th>
<th>0.382</th>
<th>0.422</th>
<th>0.495</th>
<th>0.442</th>
<th>0.474</th>
<th>0.406</th>
<th>0.469</th>
<th>0.479</th>
<th>0.383</th>
<th>1.000</th>
</tr>
</thead>
<tbody>
<tr>
<td>JBR-08.08</td>
<td>0.577</td>
<td>0.470</td>
<td>0.428</td>
<td>0.534</td>
<td>0.619</td>
<td>0.636</td>
<td>0.430</td>
<td>0.343</td>
<td>0.452</td>
<td>0.529</td>
<td>0.578</td>
<td>0.549</td>
<td>0.626</td>
<td>0.472</td>
<td>0.533</td>
<td>0.428</td>
<td>0.373</td>
<td>0.679</td>
<td>0.763</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBR-13.2</td>
<td>0.582</td>
<td>0.428</td>
<td>0.343</td>
<td>0.452</td>
<td>0.529</td>
<td>0.578</td>
<td>0.472</td>
<td>0.533</td>
<td>0.428</td>
<td>0.373</td>
<td>0.679</td>
<td>0.763</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>JGR-06.08</td>
<td>0.577</td>
<td>0.470</td>
<td>0.428</td>
<td>0.534</td>
<td>0.619</td>
<td>0.636</td>
<td>0.430</td>
<td>0.343</td>
<td>0.452</td>
<td>0.529</td>
<td>0.578</td>
<td>0.472</td>
<td>0.533</td>
<td>0.428</td>
<td>0.373</td>
<td>0.679</td>
<td>0.763</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBR-12.11</td>
<td>0.554</td>
<td>0.442</td>
<td>0.343</td>
<td>0.452</td>
<td>0.529</td>
<td>0.578</td>
<td>0.472</td>
<td>0.533</td>
<td>0.428</td>
<td>0.373</td>
<td>0.679</td>
<td>0.763</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBR-1</td>
<td>0.582</td>
<td>0.428</td>
<td>0.343</td>
<td>0.452</td>
<td>0.529</td>
<td>0.578</td>
<td>0.472</td>
<td>0.533</td>
<td>0.428</td>
<td>0.373</td>
<td>0.679</td>
<td>0.763</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBR-1</td>
<td>0.582</td>
<td>0.428</td>
<td>0.343</td>
<td>0.452</td>
<td>0.529</td>
<td>0.578</td>
<td>0.472</td>
<td>0.533</td>
<td>0.428</td>
<td>0.373</td>
<td>0.679</td>
<td>0.763</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBR-1</td>
<td>0.582</td>
<td>0.428</td>
<td>0.343</td>
<td>0.452</td>
<td>0.529</td>
<td>0.578</td>
<td>0.472</td>
<td>0.533</td>
<td>0.428</td>
<td>0.373</td>
<td>0.679</td>
<td>0.763</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GBR-1</td>
<td>0.582</td>
<td>0.428</td>
<td>0.343</td>
<td>0.452</td>
<td>0.529</td>
<td>0.578</td>
<td>0.472</td>
<td>0.533</td>
<td>0.428</td>
<td>0.373</td>
<td>0.679</td>
<td>0.763</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 4.16: Jacard’s Similarity coefficient of 16 binomial genotypes based on pooled data of all biochemical markers.**
Figure 4.16: Dendrogram depicting the genetic relationship among 16 bird species based on pooled data of all biochemical and molecular markers.
Sixteen genotypes of Brinjal was observed through molecular marker compare with the biochemical marker, as better comparison between marker study was an efficient tool for studying the genetic diversity. When pooled analysis of both the markers was done, so the molecular marker analysis showed that molecular data were more diverse and similar range of clustering pattern were observed molecular and biochemical analysis showed that molecular data were more accessions then do biochemical markers. The comparison of both and a greater range of genetic distance for discriminating among individual accessions by molecular marker yield a higher percentage of polymorphic revealed by molecular marker in the above discussion it has been concluded that data revealed.

For pooled molecular and biochemical data, coefficient range was observed 0.04 to 0.77 for molecular data and 0.45 to 0.77 for biochemical data. The similar analysis of molecular (RAPD, ISSR and SSR) markers, the similar two genotypes found in most diverse condition, in pooled among 16 genotypes. Similar clustering pattern was also observed, indicating that PLR-1 and CP-149 was the most diverse genotypes. Clustering pattern of molecular and biochemical markers could not be used in the crossing programme. Two phenogram that the two genotypes were closely similar therefore UBG-R-06-08 were closely similar (76%). It can be concluded by this molecular phenogram showed that two genotypes (UBG-R-02-11 and 0.46-1.00). Both pooled molecular and pooled biochemical and as isoenzymes have showed the highest range of genetic similarity i.e., while the genetic similarity range differed in case of isoenzymes data, approximately similar with the pooled molecular data (0.39 to 0.76). The genetic similarity ranges from 0.45 to 0.77 which was
Summary and Conclusions
SSR Primers.

(2) DNA fingerprinting through seven RAPD, 10 ISSR and 10

profiles using PAGE.

esterase, peroxidase and polyphenol oxidase, and protein

Biochemical techniques like isoenzymes viz., catalase,

with two main objectives as given below:

Based on biochemical and molecular markers was conducted

could reflect the diversity among the genotypes, the experiment

keeping this in view and to check whether this technique

for

molecular methods such as RAPD, ISSR, and SSR were sought

speciality and developmental stage. Therefore, the use of

but they are also influenced by the environmental factors, issue

are the tools to detect polymorphism among various genotypes

characteristic which is a tedious process. Biochemical markers

However, morphological grouping may be based on a
determined using morphological and agronomic characteristics.

geneic diversity in crop species including binucleate can be

2.11. The experimental material was comprised of 16 binuclal

College of Agriculture, J.A.U., Jhanginda during the year 2010.
The Biotechnology Laboratory at Department of Agri. Botany,

first biochemical and then molecular analysis was conducted in

SSR markers. The experiment was partitioned into two parts,

characterization of different genotypes of binuclear (Solanum

The present study was carried out to assess

SUMMARY AND CONCLUSIONS

CHAPTER - V
numbers of polymorphic markers. More precisely than ISSR, even RAPD also generated good
distinctive genotypes from each other as good as RAPD but through the ISSR marker was less polymorphic they can be
distinguished genotypes. Among the studied techniques, the RAPD and ISSR
investigation revealed highly diverse and different map of 16 clones
All the three techniques of DNA fingerprinting used in this

5.2 Molecular Characterization

similar dendrogram pattern as pooled enzymatic analysis.
The pooled study of isoensymes and proteins generated
two main clusters with the similarity range of 0.46 to 1.00.
all the sixteen genotypes. Pooled study of isoensymes generated
genotypes which revealed resemblance of 0.930 to 0.938 amount
12 DAG. Pooled analysis of enzymatic pattern differentiated the
average PIC value of all the isoensymes was recorded as 0.734 at
of five bands at 12 DAG in which three bands were polymorphic.
profile generated by acid phosphatase isoensymes showed total
three and four polymorphic bands at 12 DAG, respectively.
peroxidase and polyphenol oxidase isoensymes generated total of
isoensymes at 12 DAG. Out of four two were polymorphic.
A total of four bands were generated through esterase
profile generated two major clusters.

protein. Out of ten days PIC value was found about 0.925. The protein
in which all the bands were polymorphic. Seven and
at different stages of germination (7 and 14 DAG) screening
of 7 and 14 DAG. The protein profile produced total of 16 bands
12 DAG and protein analysis was done from the bulked seedlings
of bulked genotypes. The isoenzyme analysis was carried out at
isoenzymes and protein were used for the characterization

Summary and Conclusions
Studying the genetic diversity concluded that the molecular markers can be a better tool for SSR. Based on molecular data in the present study, it can be to 0.069 in RAPD, 0.170 to 0.724 in ISSR and 0.385 to 0.857 in average similarity of 16 binary genotypes was ranged from 0.188 polymorphism was detected as compared to the RAPD. The to be more effective than other techniques even a low among all the three techniques used, ISSR and SSR seem main clusters. SSR data precisely organized sixteen binary genotypes into two SSR primer was ranged from zero to 0.778. The dendrogram based on polymorphism and 3.2 fragments per primer. PIC value for each which 27 was polymorphic bands with an average of 86.0%. all the SSR primers amplified a total of 32 bands out of 3 clusters analysis revealed the two main clusters. for each primer which was ranged from 0.231 to 0.845. The upper and lower 100% polymorphic primers showed 100% polymorphism except one primer of 68 bands were obtained from the ten ISSR A total of 68 bands were amplified together in their respective sub-cluster. two main clusters that consisted of the genotypes grouped The phylogenetic tree constructed by UPGMA method generalized varying from 0.739 to 0.859 with an average of 0.807 per primer. was 95.01%. The polymorphic information content (PIC) was polymorphic. Five primers gave 100% polymorphism while two monomorphic. Five primers gave 100% polymorphism with an average of 7.14 bands per primer, while remaining three bands were out of which 47 bands were polymorphic with an average of 7.14 Seven RAPD primers amplified a total of 50 bands/alleles
Genotypes

Analyses to generate DNA fingerprinting in brinjal use primers were most useful for genetic diversity Opa-01, Opw-11, Opa-09, OBP-12 and Opc-09. Therefore, the highest polymorphism percentage with the primers viz, 2. Genetic diversity analysis through RAPD markers gave

Genetic diversity.

As under:

The conclusions drawn from the present investigation are:

The behavior of brinjal genotypes.

In our knowledge and understanding of the structure and molecular techniques for genetic analysis would lead to increase the development of specific markers. Primer designing is also a novel technique for ISSR (SCARS) can be developed from genotype specific RAPD, ISSR Nowadays, sequence characterized amplified regions are more accurate than biochemical markers. Molecular techniques of DNA fingerprinting. Thus, the molecular techniques of DNA fingerprinting were poorly distinguished genotypes precisely as compared to the biochemical techniques of 16 brinjal genotypes. However, the biochemical techniques of molecular markers resulted in developing highly diversified and amenable to amplify the data of RAPD, ISSR and SSR markers.

Among sixteen brinjal genotypes, the distinct clustering pattern was observed between the main cultivated varieties, three SSFVs, Himes and eight Germplasm lines, while amplifying the data of RAPD, ISSR and SSR markers.
as parents in future breeding programme.

other genotypes and therefore, both genotypes can be used
namely PLR-1 and GP-149 were genetically diverse from
5. Out of all the sixteen genotypes studied, two genotypes
primers can be used for diversity analysis.

as compared to RAPD and ISSR primers. Therefore, theese
Reproducibility percentage of selected primers was higher.
4. Ten SSR primers showed 84.37% polymorphism.

study in different rhizobial cultivars.

ISSR primers selected can be used further for diversity
100% polymorphism except one primer. Therefore, the
3. All the ISSR primers selected in the present study gave

Summary and Conclusions
References


Anderson, J. A., Churchill, G. A., Struebig, J. E., Tanksley, S. D. and
Biological Science. 10: 3195-3199.
using electrophoretic tuber storage proteins. Pakistan Journal of
250-252.
PAGF). Seed Science & Technology. 16: 583-599.
and Genetic Diversity Studies in Capsicum Using Seed Structure
(USA). 5: 234-235.


Agricultural Development Research, Cairo, Egypt, 13-18 February. Analytical
Cousin, B., Barsade, L., Fourth conference of agricultural
(eds) and Francis, R. R. (1993). Application of electrophoretic
Abdel-Tawab, R., M. El-Sayyed, M. El-Deby, M., E.,
Barbade, LE., R., M. El-Deby, M., E.,


Agricultural Development Research, Cairo, Egypt, 13-18 February. Analytical
Cousin, B., Barsade, L., Fourth conference of agricultural
(eds) and Francis, R. R. (1993). Application of electrophoretic
Abdel-Tawab, R., M. El-Sayyed, M. El-Deby, M., E.,
Barbade, LE., R., M. El-Deby, M., E.,


Agricultural Development Research, Cairo, Egypt, 13-18 February. Analytical
Cousin, B., Barsade, L., Fourth conference of agricultural
(eds) and Francis, R. R. (1993). Application of electrophoretic
Abdel-Tawab, R., M. El-Sayyed, M. El-Deby, M., E.,
Barbade, LE., R., M. El-Deby, M., E.,

Horticultural 107: 352-357.

Bicemical Genesics 48: 524-537.
Science. 82: 9-10.
2003-04.
Anonymous (2010). State-wise area, production and productivity of
vegetables in India. National Horticulture Board Report.

Procedure for small quantities of fresh leaf tissue.


markers. Genetics and Molecular Research. 9(3): 1568-76.

Genetic diversity of Solanum melongena


Plant Physiology. 61: 1037-1039.

in protoplasts from developing wheat bean endosperm.


Netherlands). pp. 251-274.

and Marnati, Nijmegen University Press, Nijmegen. The

Solanumaceae edited by Van Der Bie, R. C. Bentheo, G. W.

Challenge for Molecular Geneticists and Genetic Plant Breeders

and Allied Species: A New

Genetic Resources of

M. Prang, A. and Dzerojan, S. (2001). Genetic Resources of


isozyme studies of wild and cultivated Capsicum annuum


Culturre. 65: 91-107.

biotechnologies in Domesticated Plant Cell. Tissue and Organ

Ducruex, G. and Smiechowski, D. (2001). Applications of

C. I. L. Ann, V. Martin, I. K. Rajam, M. V. Seres, A.

Collombier, C., Rok, I., Kashyap, V., Roldano, G. L., Dauny, M.
Plant Genetic Resources. 6(3): 232-236.


Journal of Biochemistry and Biophysics. 7: 442-446.


Analyses of dihaploids derived from somatic hybrids
Proceeding of the National Symposium on Role of Plant
Plant Regeneration in Wild Species of Eggplant. In:
Hyderaabd, India, December 11-14. Conference on Life Sciences in Next Millennium,
Solanum melongena L.) Species for Resistance to
Kashyap, V. and Rejaim, M. V. (1999). RAPD Finger-printing of
South Campus), New Delhi, India.
hybrids between wild species and cultivated eggplant
characterization of dihaploids derived from somatic
Kashyap, V. (2002). Morphological and Molecular
Applied Genetics. 60(3): 578-583.
Solanum melongena L.) Theoretical and Applied
Solanum melongena L. (Solanaceae). Theoretical and Applied
Khaloo, C. (1993). Eggplant (Solanum melongena). In: Khaloo,


Development of SSR markers derived from SSR-enriched
Nunome, T., Negoro, S., Kono, I., Kano, M. and Myakate, K.
Neltholman, J. 1996. Eggplant (CRC Handbook of Pruit Set and
Gene and implications for broadening the breeders' gene
Diversity in commercial varieties and landraces of black
Rusirum riot resistance gene in eggplant. Theoretical and
ol of SSR, SAPIR, RAP, and SCAR linked with a
Muniru, N., Boyac, F., Cigdem, M. and Aback, K. (2008), Development
Technology. 2(4): 91-94.
chilll is revealed by RAP method. Indian Journal of Science and
Kumar, H. D. (2009). Genetic diversity in commercial varieties of
Makari, H. K., Ravikumar, Palli, H. S., Abhilash, M. and Mohan
by Wen-Hsiung Li, pp. 99-147.
Leimami, K. (1970). Cleavage of structural proteins during the

Relation in Capsicum annuum [L.] cultivars through microsatellite
Plant. 8: 1-10.

analyzed by RAPDs. Genetic Resources and Crop Evolution
of Capsicum annuum (Solanaceae) from northwestern Mexico
(2006). Genetic Structure of Wild and Domesticated Populations
A. Sanchez-Pena, P. Carmona-Tiznado, J. A. and Casas, A.
Oyama, K., Hernandez-Velarde, S., Sanchez, C., Gonzales-Rodriguez,

Breeding Science. 35: 77-83.

Characterization of Intronucleotide Microsatellites in Eggplant

and Applied Genetics. 119: 1143-1153.


Seeds Science and Technology. 35: 709-721.

International Conference. 1-4:

Genetic diversity of B. napus revealed by SSR markers. Qi-jin, W., Fu-kuan, Z., and Xing, Y. (2010). International Conference. 1-4:


International Journal of Botany and Biotechnology. 6(2): 657-666.

International Journal of Biotechnology and Biomechanics.

Genetic diversity within the genus Solanum (Solanaceae) as

using Random Amplified Polymorphic DNA (RAPD) markers.
eggplant (Solanum melongena L.) and their related wild species
Sarave, B. (2005). DNA polymorphisms in some Mau'ulian varieties of
specie. Indian Journal of Plant Genetic Resources.
eggplant, S. (1999). Status of resistant germplasm in
Sarathy, B.D., Veraprasad, K.S., Cherasabary, S.K., and
eggplant (Solanum melongena L.) and related species. Buphycra

random amplified polymorphic DNA (RAPD). ISHS Acta
Jordanian eggplant (Solanum melongena L.) Land races using
or genetic, morphological and agronomical diversity among
Age International (P) Limited, Publishers.
Phylogenetic studies in eggplant. BMC Genomics. 9:357-370.

Gene based microsatellite development for mapping and

12:1339-1348

Isolate nuclear microsatellites from plants' Molecular Biology.

Variation within Solanum sect. Patera, S. Ethephase

Accessions of other Lycopersicon species. Theoretical and Applied

Classification of Pepper (Capsicum annuum L.) Accessions
Matsuyama, R., Matsuyama, T., Sukumar, S., Okuda, N. and Kanayi, Y.
Sih, M., Galvan, C. A., Quiroa, L. S., Velez, E., Villeda, E.

165: 371-382.

Solanum commersoni accessions from Uruguay. Biphysica
Molecular marker diversity and bacterial wilt resistance in wild
Sih, M., Galvan, C. A., Quiroa, L. S., Velez, E., Villeda, E.

94.

and Ral, M. (2007). RAPD markers for hybrid seed purity testing
Sinh, P., Sing, M., Kumar, S., Kumar, R., Sinha, H., Prasanna, H.

and Ral. M. (2007). RAPD markers for hybrid seed purity testing
Sinh, P., Sing, M., Kumar, S., Kumar, R., Sinha, H., Prasanna, H.

Biochemistry. 18(2): 189-195.

Cultivars using RAPD and ISSR markers. Journal of Plant
Molecular Characterization of Brinjal (Solanum melongena L.)


of ISSR markers in the genus Lycopersicon, Euphytica


characterization of African Euphytum germplasm collection.
V. Mundala, G., Alessandro, A. D., Barchi, M., Riccardi, P., Morei,
Sunseri, R., Poligamo, G., B., Poligamo, G. B., Abba, V., Latt, C., Biscarano, V.


Solanum aegyptium L. and S. aethiopicum L. (Solanaceae) in

Gilo and Aculeating Groups into Cultivated Eggplant (S.
Persianum with resistance introduced from Solanum aethiopicum
Topfino, I., Vela, G. and Puerto, C. L. (2002a). Inheritance of
Agronomica Sinica. 35 (8): 1451-1457.

Potato cultivars (Solanum tuberosum L.) in China. Acta
Genetic diversity with SSR markers for eighty-eight approved
Liping, J. (2009). Constitution of fingerprinting and analysis of
Yanping, D., Jie, L., Chunsong, B., Shaoqiang, D., Jianpei, X., and
Vargas-Ponce, O., Perez-Alvarez, L. F., Zamora-Levares, P.
10. 503-507.
method indicating orthodonty with peroxidase, physiochemistry.
Văn Loan, L. C. (1971). Tobacco polyphenol oxidase, a specific staining
of the Institute of Technology.
Submitted to the Graduate School of Engineering and Sciences.
Tumbul, Y. (2007). Determination of Genetic Diversity Between
Tumepro, C., Menella, G., Rizza, P., D'Allessandro, A., Sichaklar, D.
Solanum melongena and
and Rottino, G. L. (2008). ISSR and Isozyme Characterization of
and Rottino, G. L. (2008). ISSR and Isozyme Characterization of
and Rottino, G. L. (2008). ISSR and Isozyme Characterization of
and Rottino, G. L. (2008). ISSR and Isozyme Characterization of
amplification. Genomics. 20: 176-83.

Hierarchicality by simple sequence repeat (SSR) anchored PCR


Applied biochemistry and biotechnology. 105: 677-687.

Characterization of acid phosphatase from garlic seedling.

import Excel file into the NTSYS program as indicated below.

When opening the NTSYS PC2 file, select 'Open Excel' from the dropdown menu. Navigate through the analysis, and ensure that matrices are needed. Run only two tools: Excel and NTSYS PC2.

The guideline to use the NTSYS program involves analyzing DNA bands and their presence or absence. The table indicates the first row as the starting point and the presence or absence of electrophoretic band patterns in the second row. The first step is to prepare an Excel file of 0 and 1 table according to the guidelines provided.
After importing the file, click save file as in the file menu and save it in appropriate location and name it e.g., here the name is given as ‘tree.NTS’.

3. Then open the NTSYS and go in to similarity for SIMQUAL (Similarity coefficient). Input tree.NTS file then select J in coefficient, and save output file by desired name e.g., simqual, in the same location and then compute. Close the window and click on clustering in the NTSYS program for subsequent analysis.
cendoroform just click on Plot tree as indicated below.

By clicking on compute the tree plot will be developed. To see the

SAHN and select FIND indices of WAAP then compute.

and input simipual file, save the file in same location with the name

4. Open clustering in NTSYSpc click on SAHN (Sahman coefficient)
5. To check the goodness of fit of the data the cophenetic coefficient (COPH) and the matrix coefficient (MxComp) are calculated. Open COPH in clustering, input SAHN file and save output file as COPH and then compute. The COPH program will calculate the data on the basis of dendrogram and compare it with the similarity coefficient so it is reverse calculation.

6. Open MxComp in graphics and input the COPH in file-1, SIMQUAL in file-2 and then compute.