MAKING SEQUENCE CHARACTERIZED AMPLIFIED REGION MARKER FOR *Lagenaria* (BOTTLE GOURD)

THESIS

SUBMITTED TO THE

NARENDRA DEVA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE (AGRICULTURE)

IN

AGRICULTURAL BIOTECHNOLOGY

BY

PRANCI TIWARI
Id. No. A-10048/17

DEPERTMENT OF PLANT MOLECULAR BIOLOGY & GENETIC ENGINEERING
Dr. RAM MANOHAR LOHIA INSTITUTE OF PLANT BIODIVERSITY AND BIOTECHNOLOGY
NARENDRA DEVA UNIVERSITY OF AGRICULTURE & TECHNOLOGY NARENDRA NAGAR (KUMARGANJ), AYODHYA-224229 (U.P.)
INDIA

2019

MASTER OF SCIENCE

IN

AGRICULTURAL BIOTECHNOLOGY

DEDICATED

To My

Beloved Parents

Mr. Ram Pal Tiwari &
Mrs. Geeta Tiwari

Pranci... .

Dr. N.A. Khan
Associate Professor

DEPARTMENT OF PLANT MOLECULAR BIOLOGY & GENETIC ENGINEERING
N. D. University of Agriculture and Technology,
Kumarganj, Ayodhya-224 229 (U.P.) India

CERTIFICATE-I

This is to certify that the thesis entitled **"Making Sequence Characterized Amplified Region marker for *Lagenaria* (Bottle Gourd)"** in the subject of **Agricultural Biotechnology** to the Narendra Deva University of Agriculture and Technology, Narendra Nagar (Kumarganj), Ayodhya (U.P.) is submitted by **Miss. Pranci Tiwari, Id. No. A-10048/17** under my supervision and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been duly acknowledged.

Narendra Nagar
July, 2019

(N.A. Khan)
Major Advisor & Chairman

CERTIFICATE-II

This is to certify that the thesis entitled **"Making Sequence Characterized Amplified Region marker for *Lagenaria* (Bottle Gourd)"** submitted for the degree of **Master of Science (Agriculture)** submitted by **Miss. Pranci Tiwari, Id. No. A-10048\17** to the Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, in partial fulfillment of the requirements for the degree of **Master of Science** in the subject of **Agriculture Biotechnology** has been evaluated satisfactory by the external examiner and approved by the student's advisory Committee after an oral examination on the same in collaboration with an external examiner.

Advisory Committee **(External Examiner)**

Major advisor & Chairman **(N.A. Khan)**
Associate Professor
Department of PMB &GE

Major **(Shambhoo Prasad)**
Associate Professor
Department of PMB & GE

Minor **(O.P. Verma)**
Assistant Professor
Department of GPB

Related Field **(R.P. Singh)**
Professor
Department of Agril. Biochemistry

Dean's Nominee **(Pratibha Singh)**
Professor
Department of Agril. Biochemistry

Dean, College of Agriculture **Head of the Department**

नरेन्द्र देव कृषि एवं प्रौद्योगिक विश्वविद्यालय, अयोध्या
NARENDRA DEV UNIVERSITY OF AGRICULTURE & TECHNOLOGY, KUMARGANJ
AYODHYA
U.P. INDIA
उत्तर प्रदेश भारत
अन्नं हि जीवानां

ACKNOWLEDGEMENT

Acknowledgement for a few might be just a trifle thing written on a piece of paper. But in its true essence it gives us an opportunity to remember and express our feelings for those whom we love and revere. Here I get a chance to express my token of thanks to people who have touched me in one way or the other by their small things. Words are not enough to express my feelings for them yet these lines are not exaggeration but feelings, which come straight from heart.

At last the moment has come to look into the deeper layers of my heart which is filled with the feeling of togetherness and loveliness; consolation and satisfaction. Some are permanent and some are momentary but both involve a number of persons to whom I acknowledge my warm regards.

*First and foremost, I would like to thank "**God**" Almighty for giving me the strength, knowledge, ability and opportunity to undertake this research duty and to persevere and complete it satisfactorily. Without his blessings, this achievement would not have been possible. It is a pleasure to thank the many people who made this thesis possible.*

*It is uniquely proud privilege to me for expressing my deep emotion & Immense gratitude **Dr. N.A. Khan**, Associate Professor, Department of Plant Molecular Biology and Genetic Engineering, and Chairman of my advisory Committee for his meticulous guidance, constant inspiration and encouragement, incessant, forbearance, peerless criticism coupled with befitting counsels throughout the investigation. I simply felt myself blessed being provided with an academic advisor like him.*

*It is a matter of great pleasure for a student to work with a intellectual who has investigative spirit, academic enthusiasm, scientific outlook, tremendous patience and special sense of devotion to his field of specialization and I am lucky enough to have **Dr. K.N. Singh**, Professor, Department of Plant Molecular Biology and Genetic Engineering, who is my advisor before being retired, for his noble guidance, untiring supervision, creative suggestions, constructive criticism and keen interest throughout my endeavor in carrying out the experiment successfully. I am extremely indebted to him for being meticulous, throughout the course of investigation & preparation of this manuscript. Throughout my thesis writing period, he provided encouragement, sound advice, good teaching, good company and lots of good ideas. I would*

have been lost without him. This research work never been completed without his precious efforts.

Words can never express the indebtedness, but I dare to take this opportunity to pay my deepest sense of gratitude with profound obeisance and heartful thanks to ***Dr. R.D.S. Yadav,*** *Professor & Head, Department of Genetics and Plant Breeding.*

I extend my warm thanks to honorary members of my advisory committee, ***Dr. Shambhoo Prasad,*** *Associate Professor, Department of Plant Molecular Biology and Genetic Engineering,* ***Dr. O.P. Verma,*** *Assistant Professor, Department of Genetic and Plant Breeding,* ***Dr. R.P. Singh****, Professor, Department of Agriculture Biochemistry,* ***Dr. Pratibha Singh,*** *Professor, Department of Agriculture Biochemistry as a valuable suggestions and comments resulting in the enrichment of this manuscript.*

I am highly thankful to ***Dr. Jeet Singh Sandhu****, Hon'ble Vice-Chancellor of the university and* ***Dr. P.K. Singh,*** *Dean, College of Agriculture for providing necessary facilities, which always filled zeal and created a conductive environment for academic activities and research at the campus I wish to record my heartiest thanks to faculty members of the Department of PMB&GE, especially* ***Dr. D.K. Dwivedi****,* ***Dr. Shambhoo Prasad****,* ***Dr. Adesh****, including non-teaching staff, Deptt. of PMB&GE.*

"Next to god, they parents" all that I am and hope to be I owe my angel parents ***Sri. Ram Pal Tiwari (Moosa Maharaj)*** *and* ***Smt. Geeta Tiwari*** *they bore me, raised me, support me, taught me****,*** *and loved me. To* ***them*** *I dedicate this thesis. I wish to thank my entire extended family Nana & Nani* ***Shri Vidhya Sagar Shukla, Smt. Vidhya Shukla*** *Uncle & Aunt* ***Sri Dilip Shukla****,* ***Smt. Poonam Shukla*** *my loving brothers & sisters* ***Alok Awasthi, Anurag Awasthi, Ashutosh Awasthi, Harshit Shukla, Nitin Awasthi, Asmit Tiwari Aviral Tiwari*** *and* ***Himanshi Shukla, Muskan Shukla, Nilakshi Mishra, Anshika Mishra*** *and my loving Bhabhi* ***Smt. Damini Awasthi*** *and my loving* ***Nephew Kanisk Awasthi*** *have always been providing me a loving environment, mental strength and courage for the successful completion of the work acknowledging them for all they have done for me will be belittling them.*

*I am overwhelmed with rejoice to avail this rare opportunity to evince my profound sense of indebtness to my super seniors****, Miss Ankita Rao, Miss Akriti Dutt, Miss Tanvi, Miss Bandana, Miss Sanghmitra, Miss Shalini, Miss Bavita, Miss Reeshu, Miss Vinita, Miss Reena Rani, Miss Shivani,***

Miss Kriti, Miss Shweta, and Dr. Ajeet Tiwari, Dr. Pradeep, Mr. Ashutosh Tiwari, Mr. Vaibhav, Mr. Sumant, Mr. Vikas, Mr. Vinay, Mr. Abhinav *for the untried help, inspiration, love affection, criticism, encouragement, constructive suggestions even under stress condition was created an impact that will form edifice to my bright future.*

I am highly thankful to my respected seniors, ***Mr. Vishwajeet Yadav, Mr. Pankaj Singh, Mr. Pravin Tiwari****,* ***Mr. Nandan Singh*** *for their untiring help, inspiration, suggestion and encouragement without which my dream would not have fulfilled.*

I have no words to express my thanks to my batch mates ***Vikas Singh, Rahul, Anurag, Mishra, Ashish Yadav, Jaswant Singh, Abhishek , Prabhat K. Singh, Vishwash Mishra, Anuj and Bhawana Mathur*** *for providing a stimulating and fun environment in which to learn and grow. I indebted to my friends* ***Diksha, Garima, Deepa, Anupama,*** *and* ***Harshit Dwivedi (Bibu)*** *for helping me get through the difficult times, and for all the emotional support, comraderie, entertainment, and caring they provided.*

Lastly and most importantly, I would like to express my special appreciation and thank to ***Dr. Hemant Kumar Yadav, Principal Scientist, Department of Crop Genetic Resources & Improvement CSIR-NBRI****,* ***Dr. Neha Singh, Department of Crop Genetic Resources & Improvement CSIR-NBRI, Dr. Ajeet Tiwari****,* ***Dr. Shivani Gurjar, Mr. Vishwajeet Yadav*** *and* ***Mr. Harshit Dwivedi (****for their extraordinary support this in thesis process and for your brilliant comments and suggestions thanks to you all.*

Equal thanks and appreciation to all members of my department ***Mr. S.P. Singh****,* ***Aditya, Ram Kishan, Keshav, Ramkumar*** *and* ***Sitapati*** *for their co-operation and encouragement at all the stage of investigation right from beginning to completion.*

It's like drop in the ocean by me all regards to God for providing me energy and patience without which it would have been none. Again I thank ***'God'*** *and my family for his blessing bestowed upon me.*

Narendra Nagar, Ayodhya **(Pranci Tiwari)**
July, 2019

CONTENTS

LIST OF FIGURES

ABBREVIATIONS AND SYMBOLS

%	Percentage
@	At the rate of
µ	Micron
µl	Microliter
^{0}C	Degree Celsius
APS	Ammonium per sulphate
C-TAB	Cetyl trimethyl ammonium bromide
DNA	Deoxyribose nucleic acid
DNTP	Deoxynucleotide triphosphate
EDTA	Ethylene diamine tetra acetate
EtBr	Ethidium bromide
g.	Gram
HCl	Hydrochloric acid
i.e.	That is
Kb	Kilo base
Ma	Mili ampere
Mg	Mili gram
ml.	Mililitre
mM	Mili molar
MR	Moderate resistance
MS	Moderate susceptible
Ng	Nano Gram
OD	Optical density

PCR	Polymerase chain reaction
pH	Power of hydrogen ion
PVP	Polyvinyl pyrollidone
RAPD	Random amplified polymorphic DNA
Rpm	Revolution per minute
TAE	Tris-acetate EDTA
TBE	Tris- boric acid EDTA
TE	Tris- EDTA
TEMED	N,N,N,N-Tetra methyl ethyelene diamine
UV	Ultraviolet
V	Volts
Xg	Revolution per minute

Chapter-I

INTRODUCTION

Bottle gourd or calabash [*Lagenaria siceraria* (Mol.) Standl.] (2n = 2x = 22), also known as opo squash or long melon, is diploid belonging to the genus *Lagenaria* of the Cucurbitaceae family with a genome size of ~334 Mb (Beevy, Kuriachan, 1996, Achigan-Dako *et al*, 2008). Bottle gourd is annual herbs (EdwinWosu and Ndukwu, 2008). The leaves of bottle gourd are alternate and variable. The flowers are unisexual and white; they are present on the same plant (monoecious). It has white pulp, often is an indehiscent gourd (hard-shelled). Bottle gourd requires a minimum temperature of 18^0C during early growth, but optimal temperature are in the range of 24-27^0C.

Dry fruits of bottle gourd can be used as containers, pipes, floats, music instruments, for medicine, artistic endeavors, etc (Heiser, 1979). Bottle gourd is also widely used as the rootstock for watermelon to defend soil-borne and low soil temperature (Lee, 1994, Yetisir, Sari, 2003). Bottle gourd synonymously called white flowered gourd or calabash gourd was one of the first plant species to be domesticated for human use, providing food, medicine and a wide variety of utensils and instruments made from the large hard-shelled fruit. Bottle gourd fruit when dry form a woody rind (exocarp) that is used mostly for the manufacture of containers (for water and food) and also musical instruments (drums and flutes), fishing floats (Heiser, 1997).

The fruits are edible and considered as a good source of vitamin C, β-carotene, vitamin B-complex, pectin and also contain highest choline level (a lipotropic factor). The seed kernels contain 45% oil and about 35% protein. Modern phytochemical screening methods have shown the presence of triterpenoid cucurbitacins B, D, G, H, fucosterol, campesterol and flavone C-glycosides in it. Bottle gourd seeds are a good source of edible protein and oil. They are an excellent source of essential fatty acids(such as omega 3 and omega 6), antioxidands, vitamins and sterols. They contain the high level of vitamin E, A and C (EL-Dengawy *et al.,* 2001; Hassan *et al.,* 2008; Hegazy and EL Kinawy , 2011). They contain the pharmaceutically active compounds used to treat acne, hyper – seborrhea, BHP, hirsutism and alopecia (Piccirilli *et al.,* 2007; Prashar *et al.,* 2014).

Singh *et al.* (2012) investigated the suitable combination of organic amendment with does of chemical fertilizers to enhance the bottle gourd production on nutrient deficient. They found that the application of vermi compost with 50% recommended dose of chemical fertilizers had a significant effect on growth and yield parameters of bottle gourd. Miroslawa and Cisowski (1995) isolated 4-C-glycosylflavone: 7-0-glucosyl-6-C-glucoside apigenin, 6-C-glucoside apigenin, 6-C-glucoside luteolin, and 7,4'-O-diglucosyl-6-C-glucoside apigenin from the plant and identified them by the spectroscopic analysis: UV, FD-MS, LSI-MS, H-NMR, C-NMR, melting point, and enzymatic hydrolysis. Moreover, using high-performance liquid chromatography, they also determined that

the flavonoids in LS fruits are mainly isovitexin, isoorientin, saponarin, and saponarin 4'-O-glucoside.

The edible portion of immature fruits of bottle gourd is about 84% per 100 g edible portion, they contain: water 95 g, vitamin A 10 IU, vitamin B^{12} 0.04 mg, niacin 0.4 mg, vitamin C 11mg, Ca 16 mg, Fe 0.4 mg, P 14 mg. The energy value is about 63 kJ/100g. The seeds contain 45% oil. The weight of 1000 seeds averages 150g. Fruits of the sweet variety contain carbohydrate (2.5%), protein (0.2%), fat (0.1%) (ether extract), fibers (0.6%), mineral matter (0.5%), calcium and phosphorous (<0.01%). Other mineral elements reported to be present include iron (0.7 mg/100 g), sodium (11.0 mg/100 g), and iodine (4.5 μg/kg). The fruit also contains 15.8 μg/g retinol. The amino acid composition of the fruit is as follows: leucines, 0.8; phenylalanine, 0.9; valine, 0.3; tyrosine, 0.4; alanine, 0.5; threonine, 0.2; glutamic acid, 0.3; serine, 0.6; aspartic acid, 1.9; cystine, 0.6; cystiene, 0.3; arginine, 0.4; and proline, 0.3 mg/g. The edible portion contains: thiamine, 44 μg; riboflavin, 23 μg; niacin 0.33 mg; and ascorbic acid, 13 mg/100 g.(Milind and Satbir 2011)

The entire plant is recognized to be beneficial in ethnic systems of medicine. The fruit is sweet, diuretic, antipyretic, antibilious, tonic for the liver, vulnerary, and antiperiodic. It can cure blood diseases in persons of pitta constitution; muscular pain and dry cough. In Punjab, the pulp is applied to the soles of the feet of those with "burning feet." The seeds are fattening, cooling, anthelmintic, and a brain tonic; they can cure cough, fever, scalding urine, and earache; they also reduce inflammation

(Unani). Their oil can be applied for headache. The rind of the fruit is good for piles, while its ash is styptic and vulnerary. The root is applied in the treatment of dropsy. .However, in many cases of oilments, it is a preferred vegetables because of its cooling effect to the stomach and easy digestibility. This species also holds promise its yet unexploited possible uses of oil and protein contents of seed (Jack, 1986). Health benefits of lauki juice promotes weight loss, helps digestion and treats constipation, treats urinary problems, treats sleeping disorders, replenishes loss of water content, keeps the heart healthy, reduces inflammation of liver.

The juice of bottle gourd is a valuable medicine for excessive thirst due to severe diarrhoea, diabetes and excessive use of fatty or fried foods. The gourd fruit juice is used in the treatment of insanity, epilepsy and other nervous diseases. The juice of the fruit is used in the treatment of stomach acidity, indigestion and ulcers. Fibre helps in preventing constipation and other digestive disorders like flatulence and piles. Bottle gourd is very valuable in urinary disorders. It should be given once daily in the treatment of burning sensation in urinary passage due to high acidity of urine. It serves as an alkaline mixture. Bottle gourd helps in overcoming jaundice and also helpful in losing weight.

The wild species of bottle gourd are confined to Africa and Madagascar (Willis, 1966. Heiser (1980) supporting the view of Whitaker and concluded Africa as place of bottle gourd .Though he further conceded that there lacks a decisive evidence to distinguish between Africa and America as the original home of this species. As said bottle

gourd is considered to be originated in Africa and America (Whitaker, 1971) and it occurs in wild form in South Africa and India. Largest variability is reported from India. The bottle gourd (*Lagenaria siceraria*) is probably one of humankinds first domesticated vegetable species, providing food, medicine and a lot more. It probably originated in Africa, from where it got distributed, perhaps by floating in sea to India, China and as far as New Zealand. The gourd is now widely cultivated throughout the tropics, especially India, Sri Lanka, Indonesia, Malaysia, the Philippines, China, tropical Africa and South America. Uttar Pradesh rank second in bottle gourd cultivation within the country. Its production is of about 422.74 (000 Tonnes) of the countries total production and share about 17.20%.

A great variability is encountered in fruit shape. They may be long, cylindrical, curved, necked, oblong, round, flat round, conical, pear-shaped club shaped etc. But bottle gourd are broadly classified as long and round types. Variability in fruit characteristics exhibited by bottle gourd has been described by Sirohi and Sivakami (1991). Most fascinating variability is encountered in its fruit shape and size. Although the crop was neglected from crop improvement point of view about three decades ago but moderately intensive conventional breeding has resulted in the development of several open pollinated and hybrid varieties in this crop in India.

India is endowed with a rich variability of bottle gourd, especially with regard to fruit characteristics (Sivaraj and Pandravada, 2005). Morphological characters are useful for characterizing genotypes against

highly heritable and stable traits. Further, association of any morphological character with desirable traits or yield components serves as a phenotypic marker in the selection process.

Narendra Shivani is a variety of Narendra Deva University of Agriculture and Technology is a selection of local germplasm. This variety has a speciality of having a log slender fruit, length of 2.0 meters. Yield potential of 1300 q/ha. With proper plant care and nutrient management single plant produces more than 200 fruits on bower. Narendra Madhuri, Narendra Rashmi, NDGB 619 are also the varieties released from NDAU&T through the selection from local germplasm. Fruit of Narendra Madhuri is round shaped which get mature at the time of mid-July to mid-august. The plant will remain in fruiting for 6 month when trained on bowers and having a yield potential of more than 1000 q/ha. The fruit of Narendra Rashmi is long bottle shaped. Its fruit is mainly cultivated in summer/rainy season. It require about 60 days for first fruit Picking and yield about 300-400q/ha. Narendra Prabha (NDBG-619) have a Cylindrical fruits, and its seeds are peculiar small conical shaped. It is an early variety, require about 55 days for first picking in spring/ summer crop and average yield of about 300 q/ ha.

We are investigating this study to find out the physiological properties of four bottle gourd varieties *viz* Narendra Shivani is long & while the other three varieties such as Narendra Madhuri is round shaped, Narendra Rashmi is long and bottle shaped and the rest one variety Narendra Prabha (NDGB-619) is cylindrical and due to the elongation of

Narendra Shivani we are also trying to find out the gene responsible for its elongation.

Objective:

In this programme we like to address this problem in *Lagenaria* and make Sequence Characterized Amplified Region marker for Shivani with following objectives:

1. Collection of round and long varieties of bottle gourd including Narendra Shivani variety.
2. DNA fingerprinting using RAPD marker and dendrogram generation.
3. Identifying bands which is specific in Narendra Shivani and also different than round one.
4. Cloning the identified band in TA cloning vector.

Chapter-II

REVIEW OF LITERATURE

An attempt has been made in this chapter to present brief account of research carried out at various aspects of the present investigation. The literature relevant to the topic **"Making Sequence Characterized Amplified Regions marker on *Lagenaria* species and its validations"**, have been collected and reviewed systematically under following headings:

2.1 Marker work in Cucurbitaceae

2.2 RAPD work in *Lagenaria*

2.3 RAPD work to distinguish specific character/trait

2.1 Marker work in cucurbitaceae

International:

Walters *et al.* (2002) found diversity in free living population of *Cucurbita pepo* (Cucurbitaceae) as assessed by random amplified polymorphic DNA. In squash (*Cucurbita pepo*), free living populations are diverse in their distributions, ecology, history, and genetic and phenotypic, compositions. Random amplified polymorphic DNA (RAPD) data was collected from 37 wild or weedy populations and 16 cultivars, which together represented all the intraspecific taxa of *C. pepo* twenty six primers yielded 70 scorable and variable markers. The presence/ absence of bands for these markers produced a data matrix which was analyzed using cluster analysis. The analysis confirmed the finding of varying

degree of gene flow from cluster into free living populations. RAPD results supported the idea that transgenic gene flow experiments with free living populations should consider using representives from each of the three free living taxa, as well as from genetically or ecological distinct populations within these taxa.

Heidelberg (2003) assessed genetic relationship in *Cucurbita pepo* (pumpkin, squash, gourd), using AFLP, ISSR and SSR markers. Forty-five accessions were compared for presence or absence of 448 AFLP, 147 ISSR, and 20 SSR bands, their genetic distances (GDs) were estimated and UPGMA cluster analysis was conducted. The results obtained from these three marker systems were highly correlated (P € 0.001). Clustering was in accordance with the division of *C. pepo* into three subspecies, fraterna, texana and pepo, with the first two less distant to one another than to the last one. Within the clusters, sub-clustering occurred in accordance with fruit shape and size. The subsp. texana cluster consisted of six sub-clusters, one each for the representatives of its five cultivar-groups (Acorn, Crookneck, Scallop, Straightneck and Ovifera Gourd) and wild gourds. Within the subsp. pepo cluster, the representatives of two cultivar-groups (Zucchini and Orange Gourd) formed distinct sub-clusters and the representatives of two other groups (Cocozelle and Vegetable Marrow) tended to sub-cluster separately from one another but formed an assemblage with the representatives of the remaining group (Pumpkin). Within-group GDs were less than corresponding between-group GDs in nearly all comparisons. The smallest-fruited accession, 'Miniature Ball', appears to occupy a genetically central position within *C. pepo*.

Levi *et al.* (2004) used forty-four randomly amplified polymorphic DNA (RAPD) markers and twenty-one anchored simple sequence repeat (ISSR) for polymorphism among heirloom watermelon cultivars *(Citrullus lanatus var. lanatus)* that had limited genetic diversity. Low polymorphism is predominant across most major linkage groups of the watermelon genome, except for one linkage group that showed high polymorphism. Four (50%) of eight markers tested for this linkage group were polymorphic among cultivars. RAPD markers produced low polymorphism, while ISSR markers were four times more polymorphic. In contrast with RAPD all 128 AFLP markers produced were polymorphic among cultivars. Thus, genetic diversity and relatedness were assessed among cultivars using 58 ISSR and 128 AFLP markers and a dendrogram was constructed using the unweighted pair-group method with arithmetic average (UPGMA). The dendrogram showed phylogenetic relationships that are consistent with parentage data known for some of the heirlooms. Result indicated that AFLP and ISSR markers produce higher resolution than RAPD markers, and provide accurate differentiation among watermelon genotypes with limited genetic diversity.

Dje *et al.* (2006) applied a molecular approach using inter-simple sequence repeat (ISSR) markers on three African edible-seeded cucurbits (*Citrullus lanatus* L. Mastsumura and Nakai, *Cucumeropsis mannii* L. Naudin and *Cucumis melo* var. *agrestis* L. Naudin). To obtain clear and reproducible bands on 1.5% agarose gel they screened 21 ISSR primers and three parameters (annealing temperature, gel tray and voltage and

running time). The resolution of 11 ISSR markers was performed, with optimal annealing temperature (TA) varying from 50 to 52^0C. The best combination to obtain clear and well-distinguished band patterns was 1.5% agarose gel with a 20-lanes tray (6 mm width) at 80 V for 5h. Applying the 11 ISSR primers on DNA extracted from an accession of *C. lanatus,* 66 bands with 4 to 11 bands per primer was observed.

Clarke *et al.* (2006) studied the origin and dispersal of the Polynesian bottle gourd and developed seven markers specific to bottlegourd (two chloroplast and five nuclear). The nuclear marker were developed using a new technique, where polymorphic Inter Simple Sequence Repeat (ISSR) markers are converted into single locus polymerase chain reaction. All seven markers were sequenced in 36 cultivars of bottlegourd from Asia, the Americas and Polynesia. The results supported a dual origin for the Americas bottlegourd, the chloroplast markers are exclusively of Asian origin, but the nuclear markers show alleles originating in both Americas bottle and Asia. Because hybridization of Polynesian bottle gourds with post European introductions cannot be excluded, ancient DNA from archaeological material will be useful for further elucidating the prehistoric movements of this species in Polynesia. This work has implications not only for the dispersal of the Polynesian bottlegourd but also for the domestication.

Xiang *et al*. (2007) used random amplified polymorphic and inter-simple sequence repeat (ISSR) molecular markers to detect the genetic diversity among 38 bittergourd (*Momordica charantia* L.).The results showed that 93 and 81 bands were obtained by RAPD and ISSR markers

amplified through 10 selected primers respectively. The PPB (percentage of polymorphic bands) in ISSR detection (61.29%) was higher than that in RAPD 50.54%.

Heikal *et al.* (2008) assessed genetic relationships among and within *cucurbita* species using RAPD and ISSR markers two molecular marker techniques, random amplified polymorphic DNA (RAPD) and inter simple sequence repeats (ISSR) were employed to identify the polymorphisms and the relationships between 14 genotypes, which belong to three different *Cucurbita* species (*C. pepo, C. moschata* and *C. maxima*). In RAPD analysis, six random primers reveled a total of 463 fragments, in which 405 (87.5%) were polymorphic. Thirty one out of 463 RAPD-PCR fragments were found to be useful a genotype specific markers. Ten genotypes out of 14 were identified by a total of 21 unique markers with the seven ISSR primers, which identified individual genotype from each other. Genotype Cm5 was distinguished from other genotypes by the presence of one unique fragment of 750 bp for primer S_1, while two ISSR primers (S_3 and S_6) identified the genotypes belonging to *C. pepo* from genotypes belonging to the other two species. The information on polymorphism using RAPD and ISSR in a set of genotypes is useful in the assessment of genetic diversity and genetic relationship and could be useful in the breeding programme.

Xiang *et al.* (2008) used Inter-simple sequence repeat (ISSR) to detect the genetic diversity among 38 bottle gourd accessions from 7 provinces in China. Twelve ISSR primers produced 96 polymorphic bands, with averaged 8 polymorphic bands each primer pair. The average

percentage of polymorphic bands was 83.5%. 38 accessions were clustered into 4 groups and 8 subgroups based on the ISSR data by the method of clustering analysis and 4 groups and 10 subgroups by the method of principal coordinates analysis. The results from ISSR molecular markers showed obvious correlation with the agronomic characteristics classification and the geographical distribution of the bottle gourd accessions.

Dje *et al.* (2010) assessed genetic diversity of African edible seeded *Citrullus lanatus* landraces using ISSR marker. The twenty ISSR primers generated 258 bands among which 252 were polymorphic (97.67%). The bands generated revealed three types of profile sharply distinct from each other with minor differences within each type. The Factorial Component Analysis and the dendrogram clearly separated the 80 individuals into three clusters corresponding to the three types of profile. Results showed that clusters were well separated from each other whereas accessions were not and suggested that high number of individuals taken into account for sampling missions and conservation strategies because accessions were not well differentiated from each other.

Sikdar *et al.* (2010) studied genetic diversity in eleven species of *Cucurbitaceae* by biochemical and molecular markers. Six enzyme systems were selected. Among 40 primers examined, 14 random amplified polymorphic DNA (RAPD) and 10 inter-simple sequences repeat (ISSR) primers were selected for the analysis. Genetic diversity generated by RAPD (100) and ISSR (100) fragments showed high

variations among the species. Jaccard similarity coefficients were used for the evaluation of pairwise genetic divergence, cluster analysis of the similarity matrices was performed to estimate interspecific diversity. Further, principal coordinate analysis was performed to evaluate the resolving power of the three marker systems to differentiate among the species.

Ilknur *et al.* (2010) studied genetic diversity of the Turkish watermelon using different *citrullus* species, by RAPD markers. Twenty two of 35 RAPD primers generated a total of 241 reproducible bands, 146 (60.6%) of which were polymorphic. Based on the RAPD data the genetic similarity coefficients were calculated and the dendrogram was constructed using UPGMA (unweighted pair group method with arithmetic average). Cluster analysis of the 303 accessions employing RAPD data resulted in a multi branched dendrogram indicating that most of the Turkish accessions belonging to *var. Lanatus* of *Citrullus lanatus* (Thunb). The genetic similarity coefficients with in the Turkish accessions were ranged from 0.76 to 1.0 with 0.94 averages indicating that they are closely related. So, results indicating that low genetic variability exist among the watermelon genetic resource collected from Turkish contrary to their remarkable phenotypic diversity.

Paris *et al.* (2010) studied AFLP, ISSR and SSR polymorphisms are in polymorphic *Cucurbita pepo.* It is extremely variable in fruit characteristics. He determined if these treatments reflect genetic relationship as viewed at the DNA level, through the use of AFLP and SSR markers. 45 accessions were compared for presence or absence of

448 AFLP, 147 ISSR and 20 SSR bands, their genetic distances were estimated and UPGMA cluster analysis was conducted. Correlation coefficients were 0.95 between AFLPs and ISSRs, 0.78 between AFLPs and SSRs and 0.77 between ISSR and SSRs all three comparisons with R<< 0.001 overall clustering and sub-clustering were much in accordance with two highly polygenic characteristics fruits shape and size.

Xu *et al*. (2011) partial sequencing of bottle gourd genome using the 454 GS-FLX Titanium sequencing platform. A total of 150,253 sequence reads, which were assembled into 3,994 contigs and 82,522 singletons were generated. The total length of the non-redundant singletons/assemblies is 32 Mb, theoretically covering ~ 10% of the bottle gourd genome. Functional annotation of the sequences revealed a broad range of functional types, covering all the three top-level ontologies. Comparison of the gene sequences between bottle gourd and the model cucurbit cucumber (*Cucumis sativus*) revealed a 90% sequence similarity on average. Using the sequence information, 4395 microsatellite-containing sequences were identified and 400 SSR markers were developed, of which 94% amplified bands of anticipated sizes. This work provides an initial basis for genome characterization, gene isolation and comparative genomics analysis in bottle gourd. The SSR markers developed would facilitate marker assisted breeding schemes for efficient introduction of desired traits.

Huanwenmeng *et al*. (2012) reported that fruit shape is one of the most important quality factors in many domesticated plants. In this study, they used bulked segregate analysis (BSA) to identify two sequence-

related amplified polymorphism (SRAP) markers linked to fruit shape in cucumber (*Cucumis sativus* L.). A segregating F2 population of 130 plants from a cross (B-2-2 x Y-3) of B-2-2 with long fruit shape trait and Y-3 with round fruit shape trait was used. Correlation and Regression analysis showed that markers ME21/ EM18M600 and ME9OD3M190 were linked closely to the allele of round at the fruit shape locus. Marker ME21/EM18M600 accounted for 33.1% of the phenotypic variation.

Semsang *et al.* (2013) reported the potential of SCAR marker for discrimination of a Thai jasmine rice (*Oryza sativa* L. cv. KDML 105) mutant, BKOS6, obtained from ion-beaminduced mutation, was evaluated. The marker was successfully used to identify BKOS6 variety and its hybrid varieties containing purple pigment accumulation in plant tissues.

Achu *et al.* (2013) studied the chemical evaluation of protein quality and phenolic compound contents of some *Cucurbitaceae* (egusi) oilseeds from different areas in Cameroon. These seeds are *Cucumeropsis mannii, Cucurbita maxima, Cucurbita moschata, Lagenaria siceraria and Cucumis sativus*. The seeds were cleaned, dried, ground and part of the powder was defatted. The defatted cakes were analysed for total and soluble nitrogen, true proteins and amino acids, while the undefatted seeds were analysed for phenolic compound contents. The defatted cakes had high total protein contents. The trichloroacetic acid soluble fraction of these proteins ranged from 25% (*C. maxima* from North West) to 94% of total proteins (*C. sativus* from Adamawa and South West), due to the post-harvest treatment of the seeds. They were rich in most essential

amino acids, giving protein digestibility, corrected amino acid scores of 0.67 for *C. sativus* and 0.48 for *C. mannii* which was for lysine, indicating that in the absence of tryptophan and methionine, lysine was the limiting amino acid in these seeds. These seeds had low levels of phenolic compounds (0.34 to 0.43%). Defatted *C. mannii* could be good for preparing infant formula, especially when mixed with soybean, in order to increase its lysine content.

Wang *et al.* (2015) described application of molecular marker in sex identification may facilitate sex determination in the seedling stage. The objective of this study was to use sequence related amplified polymorphism to identify sex linked markers in I. polycarpa and convert these markers into sequence characterized amplified region markers, which are much easier to identify. A total of 342 primer combinations were screened and 2770 bands were examined.

Maisuria *et al.* (2015) studied the chickpea (*Cicer arietinum L.*) is third most important legume crop called as poor men's meat due to good source of protein. However, the productivity of chickpea is limited due to fungal disease wilt, caused by *Fusarium oxysporum* f. sp. *ciceris*. Three markers CS-27700, UBC-8251200 and UBC-170500 were validated in fifteen diverse chickpea genotypes and found to be consistence for marker assisted characterization. These markers could be utilized in future for maker assisted breeding for wilt resistance in chickpea.

Cheng *et al.* (2016) studied sequence-characterized amplified region (SCAR) markers were further developed from high-GC primer RAMP-PCR amplified fragments from Lonicera japonica DNA by

molecular cloning. The four DNA fragments from three high-GC primers (FY27, FY-28, and FY-29) were successfully cloned into a pGM-T vector.

Mashilo *et al.* (2016) studied the Bottle gourd genotyped using 14 selected simple sequence repeat (SSR) markers. The number of alleles detected per marker ranged from 4 to 11, with a total of 86 putative alleles being amplified. Allele sizes ranged from 145 to 330 base pair (bp). Number of effective alleles (Ne) ranged from 1.58 to 6.14 with a mean of 3.10. Allelic richness varied from 3.00 to 8.90 with a mean of 5.23. Expected heterozygosity (He) values ranged from 0.37 to 0.84 with a mean of 0.65. The mean polymorphic information content (PIC) was 0.57. Jaccard's coefficient of similarity values ranged from 0.00 to 1.00, with a mean of 0.63. Analysis of molecular variance (AMOVA) revealed that 79%, 17%, and 4% of the variation in bottle gourd landraces was attributable to among landraces, within landraces, and between populations, respectively. The study established the existence of considerable genetic diversity among South African bottle gourd landraces. Unique landraces such as BG-4, BG-6, BG-8, BG-9, and BG-15 from cluster I; BG-55, BG-42, BG-57, and BG-58 from cluster II; BG-28, BG-23, BG-29, and BG-34 from cluster III were selected based on their highest dissimilarity index. These could be useful for bottle gourd breeding and systematic conservation.

National:

Staub *et al.* (2001) constructed linkage maps in cucumber (*Cucumis sativus* var. *sativus* L.) using morphological traits, isozymes,

restriction fragment length polymorphisms (RFLPs), and random amplified polymorphic DNAs (RAPDs). The lack of polymorphism in cucumber has led to the construction of relatively unsaturated maps (13- to 80-point). Amplified fragment length polymorphism (AFLP) markers to existing narrow-based (within *C. sativus*) and wide-based (*C. sativus* × *C. sativus* var. *hardwickii*) maps as added. Join map v. 2.0 was used to construct and to join these with historical maps. Narrow- and wide-based merged maps contain 255 and 197 markers, respectively, including. narrow- and wide-based merged maps contain 255 and 197 markers, respectively, including morphological traits, disease resistance loci, isozymes, RFLPs, RAPDs, and AFLPs. Condensation of total map distance occurred in merged maps compared to historic maps using many of the same markers. This is most likely due to differences in map construction algorithms. The merged maps represent the best fit of the data used and are an important first step towards the construction of a comprehensive linkage map for cucumber.

Rahman *et al.* (2003) tried to explore scientific creditability of narration of gourd in the scriptures of the world and traditional usage. The biological activities of important members of family *Cucurbitaceae* on human body have also been presented. It is emphasized that these plants are having high therapeutic values and must be consumed as daily nutrition. From the point of pharmacological function the seeds are advised for consumption, as they have more nutrition and omega fatty seeds.

Ram *et al.* (2006) studied usefulness of RAPD and facilitated molecular distinction among the five out of eight promising germplasm of bottle gourd (*Lagenaria siceraria*) where six out of 19 primers could amplify the genomic DNA. With one particular primer, the widest range for number of bands across the genotypes was 1 to 8; none of the bands was monomorphic. Leaf morphology variation (segmented type) could not be shown to be the major component of overall diversity. Search is on for molecular characterization of large number of bottle gourd germplasm and detection of at least one primer which could amplify and give dissimilar RAPD profiles in two genotypes where at least one should be PBOG 54, the segmented leaf genotype so that this technique could be extended to detect selfed seeds from crossed ones in crosses involving PBOG 54 which has segmented leaf as a marker traits.

Rathod *et al.* (2008) used Random Amplified Polymorphic DNA (RAPD) markers for assessing genetic diversity and relationships among 20 genotypes of bitter gourd (*Momordica charantia* L.) collected from different parts of India. The morphological data was recorded for their vegetative and reproductive character along with the data on diseases infesting for two successive cycles. DNAs from 20 genotypes were isolated using the CTAB method and a total of 143 polymorphic amplified products were obtained from 14 decamer primers, which discriminated all the accessions with a mean of 10.2 amplified bands per primer, 48.3% (69 bands) of which were polymorphic bands.

Behra *et al.* (2008) studied genetic diversity analysis of Indian bitter gourd (*Momordica charantia* L.) that allows for the development of

crop improvement strategies. A genetic analysis of 38 diverse Indian bitter gourd accessions was performed using 29 random amplified polymorphic DNA (RAPD) and 15 ISSR markers. RAPD primers yielded 208 amplicons of which 76 (36.5%) were distinct and repeatable providing an average of 2.6 polymorphisms per primer RAPD amplicons per primer ranged from 3 (OPE-19, OPW-09) to 15 (OPW-05), and varied in size from 200 bp to 3, 000 bp 15 ISSR primers provided a total of 125 bands of which 96 (74.7%) were polymorphic. Polymorphic ISSR markers ranged from 0 (UBC-841 to 12 (UBC-890) providing a mean of 6.3 amplicons per primer that ranged in size from 150-2700 bp. ISSR markers detected a higher level of polymorphism (74.7%) when compared to RAPD markers (36.5%). Concordance among bitter gourd accession grouping after cluster analysis was relatively high (r= 0.77) indicating that RAPD and ISSR based diversity assessments in this germplasm array were generally consistent. However, accession grouping within cluster subgroups derived from RAPD and ISSR marker analysis were dissimilar.

Kesawat *et al.* (2009) conducted the molecular markers, useful for plant genome analysis, have now become an important tool in crop improvement. The development and use of molecular markers for the detection and exploitation of DNA polymorphism is one of the most significant developments in the field of molecular genetics.

Islam *et al.* (2012) reported the DNA fingerprinting and genetic diversity analysis helps direct selective breeding and conservation of plant species. Since simple sequence repeats (SSR) markers are co-

dominant, they can predict level of genetic diversity and there by protect plant genetic resources of a region. Keeping the aforesaid rationale in mind, we worked on molecular characterization of eight cotton varieties in Bangladesh using simple sequence repeat (SSR) or microsatellite DNA markers.

Devi *et al.* (2013) described that an amplified RAPD product of 600 bp obtained in A. flavus isolates using a random primer OPB-11 was cloned in pGEMT easy vector and sequenced. This marker could also clearly distinguish A. flavus from other fungal plant pathogens, including different Aspergillus spp.

Nagaraja *et al*. (2013) analysed the Sr2 and Sr22 gene specific SSR markers amplified the gene specific or expected allele in both Sr2 and Sr22 carrying and non-carrying cultivars. SCAR marker, SCSS30.2 specific to Sr31 gene amplified the gene specific fragment in wheat cultivar UP 2338 confirming the presence of Sr31.

Shivakumar *et al*. (2014) described DNA fingerprint of a particular crop enables the researchers to pinpoint specific fingerprint and accurately identify seed varieties. The ability to identify seed varieties will make the test important to guaranteeing the authenticity of a crop being purchased.

Nagendran *et al*. (2016) described that Bottle gourd plants showing disease symptoms of mosaic mottling, chlorosis and yellowing of leaves from two different locations of Tamil Nadu were found to be infected with a Begomovirus through PCR assay with universal primer

pair (Deng 540/541). In BLAST analysis, it is identified as Tomato leaf curl New Delhi virus (ToLCNDV). Additionally amplified complete coat protein gene using another primer pair GK ToLCV F/R were sequenced and deposited to the GenBank database (TN MET BoG2 - KM275606; TN NGK BoG1 - KM275616). The nucleotide of TN MET BoG2 and TN NGK BoG1 had maximum identity of 94 % and 98% towards the ToLCNDV reported from Spain and Asian countries respectively. In the phylogenetic analysis, TN NGK BoG1 clustered with ToLCNDV isolate infecting Parthenium whereas TN MET BoG2 clustered with isolates infecting cucrbitaceous crops from Spain. As per our knowledge this is the first confirmed molecular evidence for the occurrence of Tomato leaf curl New Delhi virus on bottle gourd in Tamil Nadu.

2.2 RAPD work in lagenaria

International:

Garica *et al.* (2000) used three different types of molecular markers, RAPD, AFLP and RFLP were used to measure genetic diversity among six genotypes of *Cucumis melo* L. Each line represented a different melon genotype: Piel de Sapo, Ogen, PI161375, PI414723, Agrestis and C105. A number of polymorphic RAPD, AFLP and RFLP bands were scored on all materials and the genetic similarity measured. Clustering analysis performed with the three types of markers separated the genotypes into two main groups: (1) the sweet type, cultivated melons and (2) the exotic type, not cultivated melons. While the data obtained suggested that all three types of markers are equally informative, AFLPs showed the highest efficiency in detecting polymorphism.

Walters *et al.* (2001) described the morphological analyses and archaeological evidence suggest the oceanic dispersals of wild bottle gourd fruits from Africa to Asia and the Americas by 10000–15000 B.C. To clarify the evolutionary histories of bottle gourd landraces and cultivars, 64 random amplified polymorphic DNA (RAPD) markers representing 30 primers were examined in 31 landraces and 43 cultivar accessions of *L. siceraria*, as well as in a wild relative, *L. sphaerica*. Principal component analyses of the correlation matrices of the band presence/absence data.

Denna *et al.* (2004) gave genetic assessment of wild bottle gourd from Zimbabwe. This African native reaches Asia and the Americas 9000 years ago, probably as a wild species whose fruits had floated across the seas. Few wild populations *L. siceraria* have been found during recorded history and none have been verified or studied in detail. In 1992, Mary Wilkins-Ellert discovered an unusual free living plant of *Lagenaria* in a remote region of Southeastern Zimbabwe. Her morphological observation during several plantings of the collected seed as well as results from two genetic analysis (random amplified polymorphic DNA chloroplast sequencing), indicated that the Zimbabwe collection is part of a genetically distinct and wild lineage of *L. siceraria*.

Yasuyoki *et al.* (2005) investigated gene diversity of Kenyan landraces of the white flowered gourd (*Lagenaria siceraria*), which exhibit tremendous morphological variation. RAPD analyses were performed on 53 landraces of the cultivated species *L. siceraria* and 42

accessions of three wild species (40 *L. sphaerica*, *1 L. abyssinica* and *1 L. breviflora*). A total of 432 polymorphic bands were detected using 54 primers. The four species were clearly differentiated from one another. Intra specific variations were investigated with *L. siceraria* and wild relative *L. sphaerica.* Bitter landraces showed two specific RAPD band. Progeny plants derived from a common mother in *L. siceraria* showed a low level of segregation RAPD pattern showed their inherent traits. The wild relative *L. sphaerica* showed higher level segregation. The morphological diversity observed among landraces of *L. siceraria* is the result of human selection their genetic identities are maintained by inbreeding probably resulting from frequent self pollination.

Kobayashi *et al.* (2007) studied classification of bottle gourd varieties by RAPD analysis and cluster analysis to select the varieties to be preserved, YUUGAO stored in the selection of varieties for the purpose of nurturing Tochigi prefecture, including varieties 5 YUUGAO 14 breeds and varieties is a variant of YUUGAO. 10 varieties of gourd varieties for a total of 24 RAPD analysis using cluster analysis and tried to classification. As a result, 44 RANDAMUPURAIMA using 31 different types of DNA markers were obtained. A total of 24 bottle gourd varieties including 5 varieties bred by Tochigi prefecture were analyzed. Forty four primers produced 31 scorable, variable RAPD markers with in bottle gourd varieties. Only 4 polymorphic markers were produced in 10 varieties derived from Tochigi prefecture. These result suggested that these 10 varieties are closely related with each other, but not with the introduced varieties.

National:

Trivedi *et al.* (2015) studied the growth contributing characters of biofield treated bottle gourd (*Lagenaria siceraria*) and okra (*Abelmoschus esculentus*) seeds. The variability in growth contributing parameters were studied and compared with their control. Further the level of glutathione (GSH) in okra leaves, along with DNA fingerprinting in bottle gourd were analyzed using RAPD method. The vegetative growth of okra plants after biofield energy treatment was found to be stout with small canopy, strong steam, and more fruits per nodes, that contributed high yield as compared with the control. However, endogenous level of GSH in the leaves of okra was increased by 47.65% as compared to the untreated group, which may suggest an improved immunity of okra crops. Besides, the DNA fingerprinting data, showed polymorphism (42%) between treated and untreated samples of bottle gourd. The overall results suggest that the biofield energy treatment on bottle gourd and okra seeds, results an improved overall growth of plant and yield, which may enhance flowering and fruiting per plant. Study results conclude that the biofield energy treatment could be an alternate method to improve the crop yield in agricultural science.

Rai *et al.* (2017) described stem rust resistance gene Sr26 imparts resistance to all the virulent pathotypes of stem rust (*Puccinia graminis f. sp. tritici*) including a new race of pathogen named TTKSK (syn. Ug99). The Random Amplified Polymorphic DNA (RAPD) marker was converted into sequence characterized amplified region (SCAR) marker.

The marker was validated in another segregating population and 45 wheat genotypes including carrier and non-carriers of Sr26 gene.

2.3 RAPD work to distinguish specific character/trait

International:

Yang *et al.* (2002) studied cloned fragments from improved randomly ampified polymorphic DNA (RAPD) marker. The specific RAPD bands of D. logan cultivars from Guangxi, with size ranging from 500 to 900 bp were gel purified, cloned and sequenced.

Ferrial *et al.* (2004) studied genetic variability in pumpkin (*Cucurbita maxima*) using RAPD markers. The Centre for Conservation and Breeding of Agricultural Biodiversity (COMAV) holds a collection of more than 900 accessions of these 5 species (4). 8 accessions selected from a previous morphological characterization following the descriptor for cucurbita of the IPGRI (3), were used. These accessions displayed different levels of variability concerning fruit morphological traits. The accessions CM, V_1, V_2 and AN_2 are very uniform, with a granular skin texture, grey colour and variable ribs. The 11 primers used provided a total of 43 bands 32 of them were polymorphic (74% polymorphism).

Dhanya *et al.* (2005) studied the development of specific, sensitive and reproducible Sequence Characterized Amplified Region (SCAR) markers to detect these adulterants in traded turmeric powder. Two putative RAPD markers, 'Cur 01' and 'Cur 02' generated by primer OPA 01 and OPE 18 were identified as *C. zedoaria/C. malabarica* specific by comparative RAPD analysis of genuine turmeric and marker samples of

turmeric powder, *C. zedoaria* and *C. malabarica*. These specific RAPD markers were cloned and sequenced.

Hoque and Robbani (2009) assessed genetic relationship among landraces of Bangladesh ridge gourd (*Luffa acutangula* Roxb.) by using RAPD markers. Twenty eight accessions were studied. Four selected decamer primers out of sixteen screened, could generate a total of 27 RAPD fragments of which 22 were polymorphic (81.5%). The bands ranged from 50 to 1500 bp in size. Genetic variation statistics for all loci estimated the average gene diversity (h) value as 0.278 and the shannons information index (I) as 0.415. Dendrogram based on unweighted pair group method with arithmetic averages (UPGMA) segregated the accessions into 5 clusters.

Najafiniya *et al.* (2011) reported specific random amplified DNA (RAPD) markers which identified in four pathogenic group I, II, III, and IV were cloned into P-Teasy vector. The specific SCAR primer and PCR technique developed in this research easily detect and differentiate isolate of each *F. oxysporium f.* sp. *cucumerinum* pathogenic group.

Devaiah *et al.* (2011) described DNA-based markers have been developed to distinguish *I. mauritiana* from the other Vidari candidates.A putative 600-bp polymorphic sequence, specific to *I. mauritiana* was identified using randomly amplified polymorphic DNA (RAPD) technique. Furthermore, sequence characterized amplified region (SCAR) primers (IM1F and IM1R) were designed fromthe unique RAPD amplicon. The SCAR primers produced a specific 323-bp ampliconinauthentic *I. mauritiana* and not in the allied species.

National:

Sivaraj and Pandravada (2005) reported that the bottle gourd (*Lagenaria siceraria*) is a cucurbitaceous vegetable for which India is the secondary center of diversity and endowed with rich variability especially in the fruit characters. This paper documents the available fruit diversity in the bottle gourd germplasm collected from the tribal communities, *viz*., Koyas, Gutti Koyas, Lambadas, and other primitive tribal groups living in the Telangana region. The collected germplasm (54 accessions) showed immense variation in the qualitative characters of fruit such as shape, luster, blossom- end, ridges, etc. Wide range of variability was also recorded in the quantitative traits for several fruit and seed characters, viz., fruit length (13.3–83.9 cm), fruit width (7.9–34.4 cm), fruit circumference (20.5–98.0 cm), seed length (11.3–21.0 mm), seed width (5.8–9.2 mm), seed thickness (2.5–3.7 mm), and 100-seed mass (7.6–31.8 g).

Sadia *et al.* (2012) conducted the DNA fingerprints of four rose species, Rosa centifolia, R. Gruss-an-Teplitz, *R. bourboniana*, and *R. damascena*, were developed using RAPD-PCR.

Chapter-III

MATERIALS AND METHODS

3.1 Plant material:

A total of four bottle gourd (*Lagenaria siceraria)* genotypes were selected for DNA amplification using RAPD markers and making the SCAR marker for long specific variety. All the four bottle gourd (*Lagenaria siceraria)* genotypes were obtained from net house in Department of PMB & GE and Main Experiment Station, Department of Vegetable Science, of N.D. University of Agriculture and Technology Kumarganj Ayodhya and there description has been provided in Table 3.1

Table-3.1 List of bottle gourd genotypes used in the DNA isolation.

S. No.	Name of Variety	Pedigree	Special Features	Year of Release
01.	Narendra Shivani	Selection from local germplasm	Prolific bearer very long slender fruits exceed the length of 2.0 meters. Yield potential of 1300 q/ha. With proper plant care and nutrient management single plant produces more than 200 fruits on bower.	2007

02.	Narendra Madhuri	Selection from local germplasm	Rounded fruits, appropriate time of planting is mid-July to mid-August. With proper plant care and nutrient management, the plants trained on bowers remain in fruiting for about 6 months. Yield potential of more than 1000 q/ha.	2007
03.	Narendra Rashmi	Selection from local germplasm	Fruits are long bottle shaped. Summer type bottle gourd, suitable for cultivation in summer and rainy seasons. Requires 60 days for first fruit Picking. Fruits yield 300-400q/ha.	2001
04.	Narendra Prabha (NDBG-619)	Selection from local germplasm	Cylindrical fruits, peculiar small conical seeds, an early variety, 55 days for first picking in spring/ summer crop an average yield 300 q/ ha.	2009

3.2. Collection of round and long varieties of bottle gourd including Shivani variety:

Fig 3.2.1 shows the seed of bottle gourd varieties Narendra Shivani, Narendra Rashmi, Narendra Madhuri and Narendra Prabha (NDGB 619). The seed of Narendra Shivani and Narendra Rashmi was light colour, long in size while Narendra Madhuri and Narendra Prabha seed were dark in colour and long in size.

Fig 3.2.2 shows the leaves of bottle gourd. They were approximately similar in colour, shape and size.

A. Narendra Madhuri

B. Narendra Rashmi

C. Narendra Shivani

D. Narendra Prabha

A. Narendra Rashmi

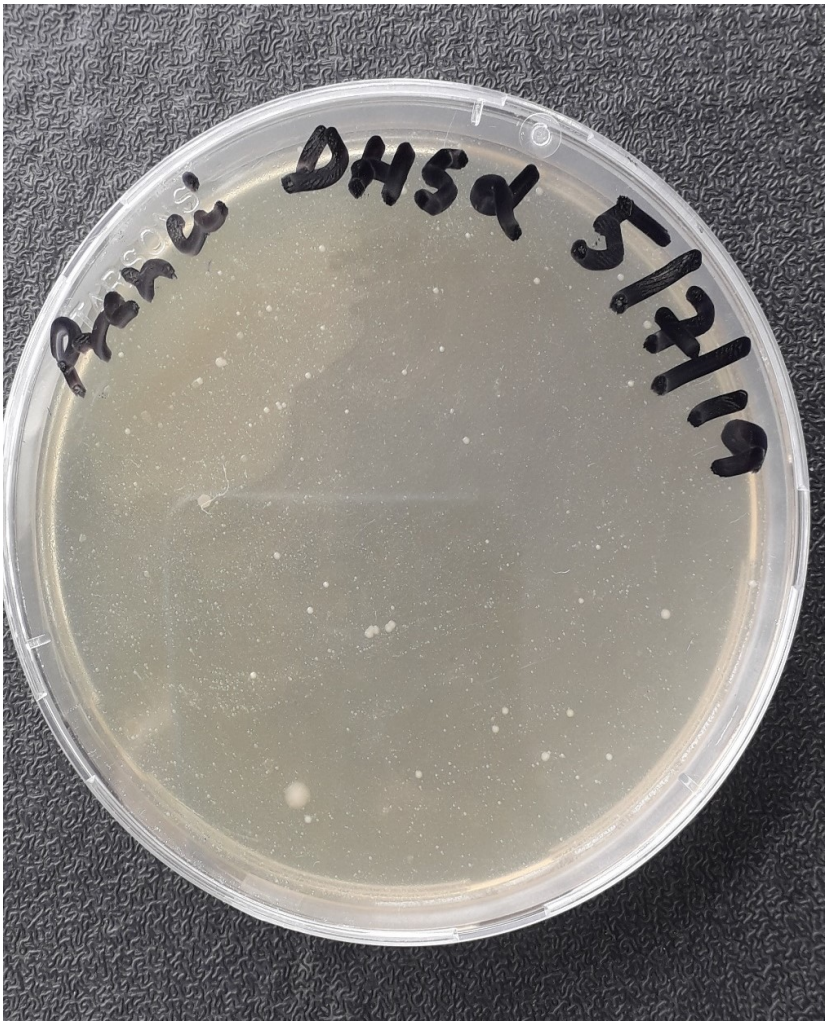
B. Narendra Shivani

c. Narendra Prabha

D. Narendra Madhuri

3.2.4 *E. coli* Host:

For transformation in *E. coli* to cloned vector, DH5α was used. Plate containing DH5α is shown in figure 3.2.4



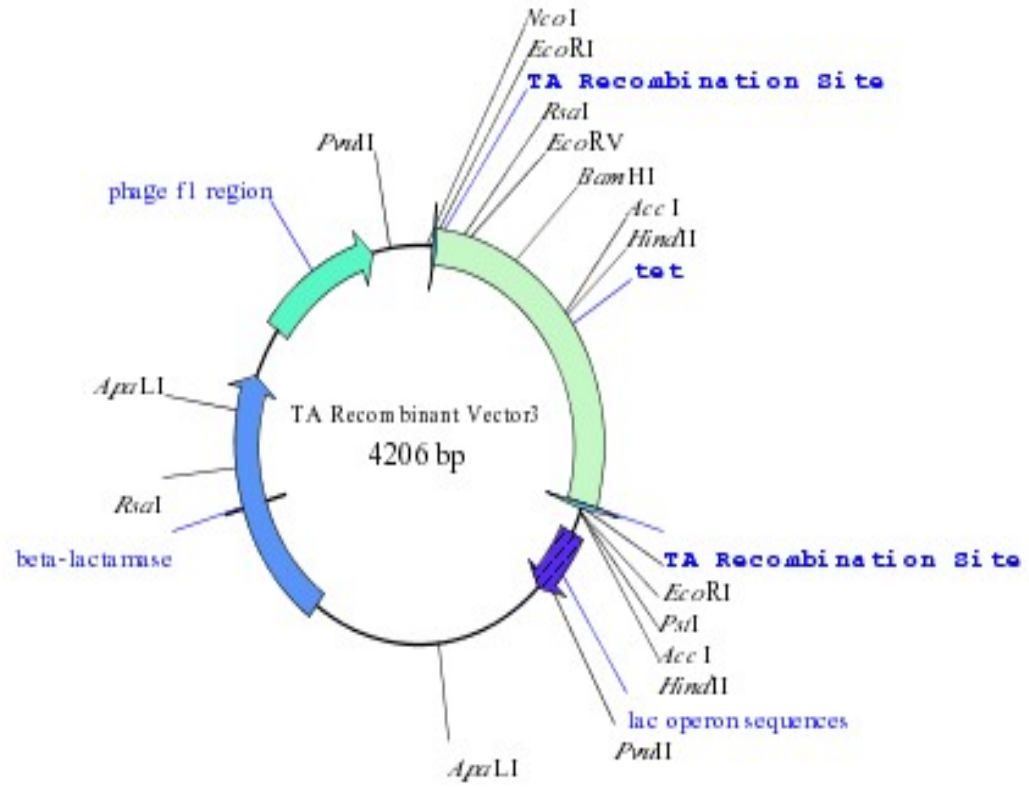
Colony of DH5α was appeared in LB Agar plate after quadraplating as seen in figure .

3.3 Primers used for PCR amplification:

S.No.	Primer	Sequence
1.	OPB 13	5'TTCCCCCGCT3'
2.	OPK 11	5'AATGCCCCAG3'
3.	OPA 19	5'CAAACGTCGG3'
4.	OPA 13	5'CAGCACCCAC3'
5.	OPO 12	5'CAGTGCTGTG3'
6.	OPB 10	5'CTGCTGGGAC3'
7.	OPB 18	5'CCACAGCAGT3'
8.	OPO 11	5'GACAGGAGGT3'
9.	OPC 15	5'GACGGATCAG3'
10.	OPO 10	5'TCAGAGGCGC3'
11.	OPK 17	5'CCCAGCTGTG3'
12.	OPB 08	5'GTCCACACGG3'
13.	OPB 11	5'GTAGACCCGT3'
14.	OPN 07	5'CAGCCCAGAG3'
15.	OPA 17	5'GACCGCTTGT3'
16.	OPC 07	5'GTCCCGACGA3'
17.	OPN 01	5'CTCACGTTGG3'
18.	OPY 19	5'TGAGGGTCCC3'
19.	OPY 18	5'GTGGAGTCAG3'
20.	OPF 14	5'TGCTGCAGGT3'
21.	OPF 13	5'GGCTGCAGAA3'
22.	OPF 06	5'GGGAATTCGG3'
23.	OPF 17	5'AATTTGGGAA3'
24.	OPY 15	5'AGTCGCCCTT3'
25.	OPJ 08	5'CATACCGTGG3'
26.	OPZ 03	5'CAGCACCGCA3'
27.	OPD 08	5'GTGTGCCCCA3'
28.	OPY 02	5'CATCGCCGCA3'
29.	OPY 14	5'GGTCGATCTG3'
30.	OPJ 13	5'CCACACTACC3'
31.	OPY 01	5'GTGGCATCTC3'
32.	OPY 09	5'GTGACCGAGT3'

3.4 Plasmid:

Fig 3.4.1 shows the TA cloning vector.



3.5 DNA isolation from young leaves of bottle gourd:

In the present study the following protocols with slight modifications have been used to extract total genomic DNA from the leaves of bottle gourd (*Lagenaria siceraria*) under optimized conditions. The protocols were as the method described by Murray and Thompson (1980).

3.5.1.Reagents and Chemicals required for DNA Isolation

Stock solution (For 100 ml):

1M Tris (pH 8.0)	12.14 g
0.5 M EDTA	18.224 g
5 M NaCl	29.22 g

Extraction Buffer:

C-TAB	3g
1 M Tris(pH 8.0)	10 ml
0.5 M EDTA	4 ml
5 M NaCl	28 ml
PVP	2 g
2 β- Mercaptoethanol	35 µL

Maintained final volume 100 ml (pH of EDTA was adjusted to 8.0 with NaOH pellets. while the pH of Tris was adjusted to 8.0 by dilute HCl).

70% Ethanol	**100 ml**
Absolute ethyl alcohol	70 ml
Distilled water	30 ml

Chloroform: Iso amyl alcohol (24:1)

Chloroform	96 ml
Isoamylacohol	4 ml
Total volume	100 ml

TE Buffer	**(100 ml)**
1 M Tris	1ml
0.5 M EDTA	200 µl

Maintained total volume 100 ml by distilled water and pH of TE buffer was adjusted to 8.0.

Ethidium Bromide

Ethidium bromide	0.1 g
Distilled water	10 ml

It was stored in dark bottle at room temperature

Loading dye	**(10 ml)**
100% Glycerol	7 ml
0.5M EDTA	3ml
Total volume	10 ml

Added a pinch of Bromophenol blue (0.01 g) in above and 10 ml volume was adjusted.

TAE Buffer (1X TAE per liter)

Tris	6.304 g
Glacial Acetic acid	1.14 ml
0.5 M EDTA	2 ml

Total volume was made up to 1000 ml. The pH of TAE buffer was adjusted to 8.0.

RNase A (10 mg/ml)

Dissolved Bovine pancreatic RNase A in 10 mMTris-Cl pH-7.5, 15 mMNaCl. Heated at 100°C for 45 min. cooled down to room temperature. Aliquots were stored at -20°C.

3.5.2 Method of DNA isolation:

a) 500 mg fresh leaves of each sample of bottle gourd were taken and ground in liquid nitrogen with the help of mortar and pestle.

Powdered leaves were transferred into centrifuge tube. Add 4ml extraction buffer in centrifuge tubes and were heated in water bath at 65^{o}C for 1 hour.

b) After that centrifuge tubes were cooled at room temperature and 3 ml of chloroform: iso-amyl alcohol (24:1) was added.
c) Mixed properly by inverting centrifuge tubes 20-25 times.
d) Solution Centrifuge tubes were centrifuged at 6,000 rpm for 15 minutes at 4^{o}C temperature.
e) Supernatant was transferred in fresh eppendorftubes tubes.
f) In supernatant, 5µl of 5 M NaCl and equal volume of iso-propanol were added and stored it at 4^{o}C for overnight.
g) Next day, eppendorf tubes were centrifuged at 10,000 rpm for 20 minutes. Supernatant was discarded and pellet was washed with 70% ethanol.
h) Pellet was re-suspended in 50µl of TE buffer and stored at 4^{o}C.

3.5.3.DNA Purification:

The genomic DNA isolated by the above methods which contained some amounts of RNA and proteins as contaminants. The RNA contamination was removed by treating isolated DNA with DNase free RNase. Thereafter additional de-proteinization steps were followed to remove RNase. These steps eliminated most of the part of protein contamination.

Procedure:

a) Bovine pancreatic ribonuclease A was prepared at a concentration of 10 mg/ml in NaCl + Tris-HCl, 2 µl RNase added in each vial.

b) DNA was heated for 40 min at 37°C.

c) Equal volume of chloroform was mixed gently and centrifuged at room temperature for 15 min. at 13000 rpm.

d) Upper aqueous layer was taken out in eppendrof tubes. Added equal volume of chloroform and phenol (1:1), mixed gently with inversionand centrifuged for 15 min at 13000 rpm at room temperature.

e) Upper aqueous layer was taken out in eppendrof tubes. Added equal volume of chloroform and phenol (1:1) mixed gently with inversion and centrifuged for 15 min at 13000 rpm at room temperature.

f) Upper aqueous layer was taken out in eppendorf tubes mixed equal volume of phenol and shaked vary gently, centrifuged at room temperature 13000 rpm for 15 min.

g) Upper aqueous layer was taken out in new eppendrof tube. Added 0.7 volume of isopropanol mixed by inversion and incubated at -20°C for 4 hours, centrifuged 13000 rpm for 15 min at 4°C.

h) The DNA pellets were washed with 70% ethanol for 5 min and pelleted by centrifugation and kept for drying at 37°C.

i) 100 µl of milliQ water were added after proper drying and left overnight at 4°C to dissolve.

3.5.4 Agarose gel electrophoresis:

Gel electrophoresis of the genomic DNAs was carried out for qualitative estimation of samples prepared. A good DNA preparation appeared as a single band. A submarine horizontal agarose slab gel

apparatus (Bangalore Genei) as described by Sambrook *et al.* (1989) was used. Agarose (0.8% W/V) was suspended in 0.5 x TAE buffer and boiled for 20 min, to dissolve it. After cooling, it was poured in an electrophoresis tray and allowed to solidify at room temperature. Slots were made by fixing a slot former (comb) over the tray, prior to pouring the agarose. The comb was removed after the agarose and gel was transferred to an electrophoresis tank containing 1X TAE buffer. Mixed 2 µl of loading dye with 3µl DNA sample. DNA sample was loaded in slot. Gel was run at constant voltage (40 V) for three hours. After completion of electrophoresis, DNA bands were visualized on trans illuminator and photographed has been taken on gel documentation system (Alpha Digidoc) USA.

3.5.5 Quantification of DNA:

a) Taken 1 ml TE buffer in a cuvette and calibrated the spectrophotometer at 260nm as well as 280 nm wave lengths.
b) Added 2 to 5 µl of DNA mixed properly and recorded the optical density (O.D.) at both 260 and 280 nm.
c) Estimated the DNA concentration employing the following formula

$$\text{Amount of DNA (µg/µl)} = \frac{\text{(OD) 260 x 50 x dilution factor}}{100}$$

3.6 Dilution of DNA for PCR

The concentrated genomic DNA stock was diluted with doubled distilled water for used in RAPD amplification.

3.6.1 Dilution and Standardization of RAPD primers:

RAPD primers dilutions were done depending on concentration made such that 1 µl diluted has 100pg.

DNA amplification and visualization

3.6.2 PCR reaction preparation:

For each sample the following chemicals are used:

Chemical	**Volume**	**Concentration**
DNA	1µL	
Taq polymerase	0.250 µL	1.25U
Taq buffer	4 µL	5 X
$MgCl_2$	3 µL	1.0-4.0 mM
dNTP	1 µL	
Primer	1 µL	10 pmol.
Double distilled water	9.75 µL	
Total	20 µL	

The cocktail so prepared for PCR reaction was added to each 1 µL DNA sample for different varieties. After this step, the sample was loaded into the PCR machine and run accordingly with desired programmed.

3.7 PCR amplification for RAPD

The amplification of DNA sample with RAPD primer was done in a DNA thermo-cycler with following programmed:

Segment	Temperature (°C)	Duration (minute)	Activity	Cycles
1	94°C	3	Denaturation	1
2.1	94°C	1	Denaturation	40
2.2	37°C	1	Annealing	
2.3	72°C	2	Extension	
3	72°C	10	Extension	1
4	4°C	Forever	Storage	

3.8 Cloning of the PCR product into T-vector

The purified DNA fragments were ligated to TA cloning vector. The ligation mixture along with linearized vector and amplicon DNA were mixed in 0.5 ml micro-centrifuge tubes and incubated at 16°C overnight.

Component	Quantity taken
Purified amplified products	5 µl
PCR cloning vector	1µl
2X Instant ligation buffer	5 µl
Instant T4 DNA ligase	1µl
Distilled water	8µl
Total volume	20µl

3.8.1 Preparation of competent cells:

The competent cells of *E. coli* (DH5α) were prepared by following the protocol mentioned by Sambrook *et al*. (1989) with minor

modifications underaseptic and refrigerated condition. An isolated colony from *E. coli* DH5α plate was inoculated into 5 ml Luria broth and incubated at 37°Covernight at 200 xg. The next day, the culture was diluted to 1:100 using Luriabroth *i.e*., 0.5 ml of culture was added to 50 ml of Luria broth. It was incubated for 2-3 hours till it attained an OD of 0.3 to 0.4 at 600 nm. The culture was chilled in ice for 30 min., and 25 ml of culture was dispensed into two centrifuge tubes of capacity 50 ml. The cells were pelleted at 6000 xg for 5 min. The supernatant was discarded and pellet was suspended in 12.5 ml of ice-cold 0.1 M calcium chloride. The centrifuge tubes were again kept in ice for 45 min. and later centrifuged at 4000 xg for 10 min. The pellet was dispensed in 1 ml of 0.1M $CaCl_2$. About 200 µl of cells were distributed to each chilled 1.5 ml microcentrifuge tubes and immediately used.

3.8.2 Transformation of *E. coli* (DH5α) and blue white selection:

About 100 µl of freshly prepared competent cells were taken in a chilled centrifuge tube and 5 µl of ligation mixture was added and mixed gently. The mixture was chilled in ice for 45 min. and heat shock was given by shifting the chilled mixture to preheated 42°C water bath for exactly 2 min., then the tubes were immediately transferred to ice for 5 min. To this, 800 µl of Luria broth was added and incubated at 37°C at 200 xg for 45 min. to allow bacteria to recover and express the antibiotic marker encoded by the plasmid. The culture was centrifuged at 10,000 xg for 1 min. and about 700 µl of supernatant was discarded and the pellet was dissolved in remaining supernatant and spread on the plates having Luria agar with Ampicillin (100 mg/ml) and incubated overnight at 37°C

for colonies to appear. The recombinant clones were identified by blue/white assay.

After incubation only white colonies, having recombinant vectors were picked up and streaked on plates having Luria agar with Ampicillin (100 µg/ml), X-gal (50 µg/ml) IPTG (Isopropyl 1-ß, D-1 thiogalactopyranoside) (200 mg/ml) and incubated at 37°C overnight.

3.9 Dendrogram Preparation

All the eight primers bands for all varieties were scored. Where the band was present 1 was given, where the band was not present 0 was given and then using NTSYS program dendrogram was generated.

Chapter-IV

EXPERIMENTAL FINDINGS

The experimental findings of present work of "Making SCAR marker for Lagenaria (Bottle gourd)" are listed below with five main sub headings:

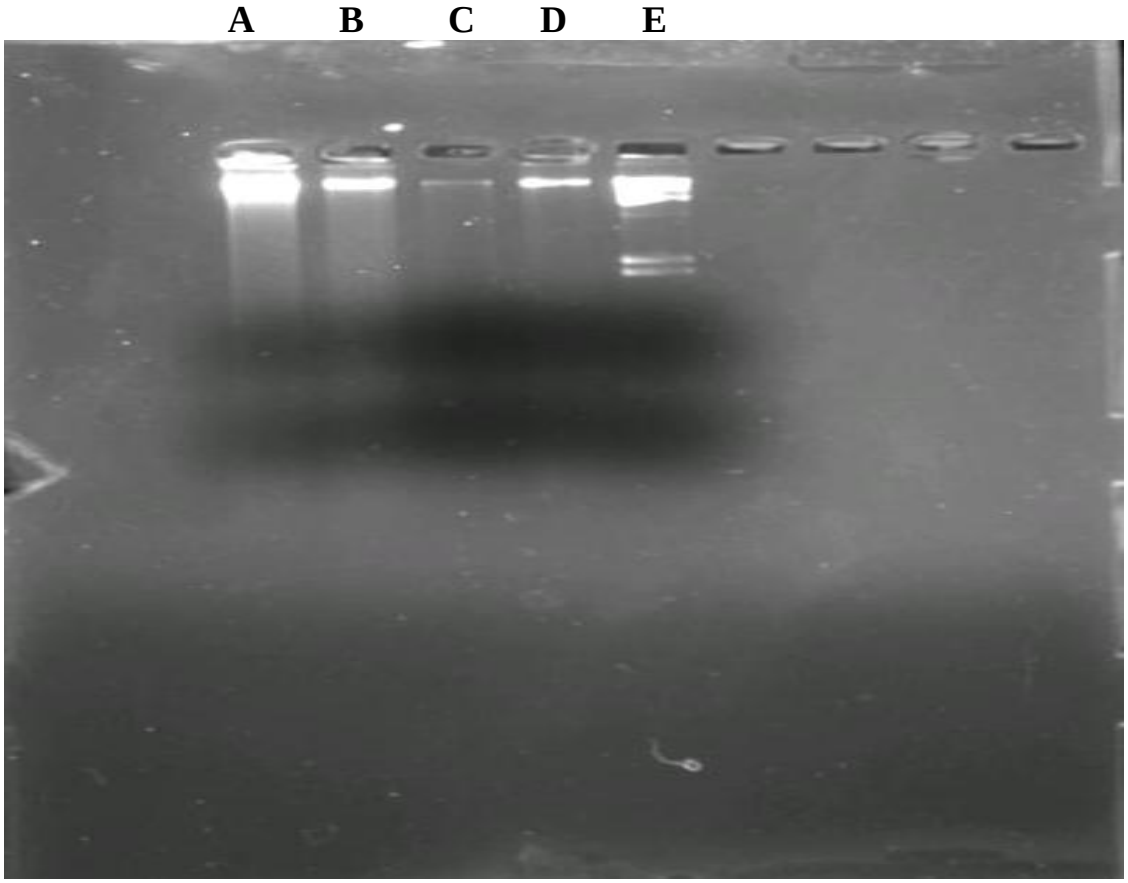
1. Collection of round and long varieties of bottle gourd including Shivani variety.
2. DNA isolation from leaves of bottle gourd.
3. PCR amplification using RAPD markers.
4. Dendrogram generation using NTSYS-pc.
5. Cloning in long specific DNA in TA Cloning vector.

4.1. Collection of round and long varieties of bottle gourd including Shivani variety:

The seeds of Narendra Shivani and Narendra Rashmi were light colour, long in size while Narendra Madhuri and Narendra Prabha seeds were dark in colour, and long in size. As shown in figure. (3.2.1).

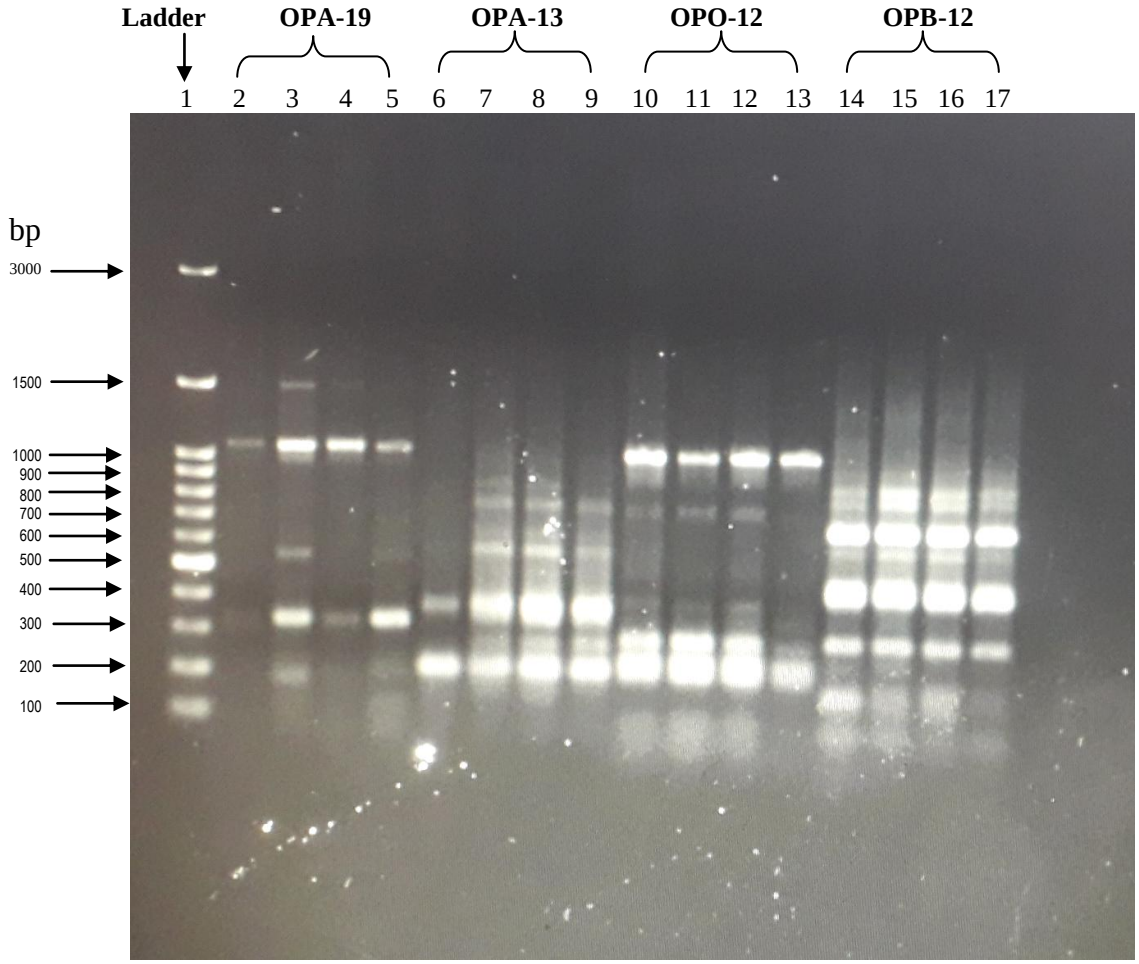
The leaves of bottle gourd were approximately similar in colour, shape and size. The fruit of bottle gourd were morphologically different. Narendra Shivani was very long, Narendra Rashmi was long, Narendra Madhuri was round shaped and Narendra Prabha was drum shaped. The flowers of bottle gourd varieties were whitish colour in all. As shown in figure. (3.2.2).

4.2 DNA isolation from leaves of bottle gourd:



DNA was in the teethed form. It was also in intact form. There was no sharing. Quality and quantity was different in all.

4.3 PCR amplification using RADP marker:



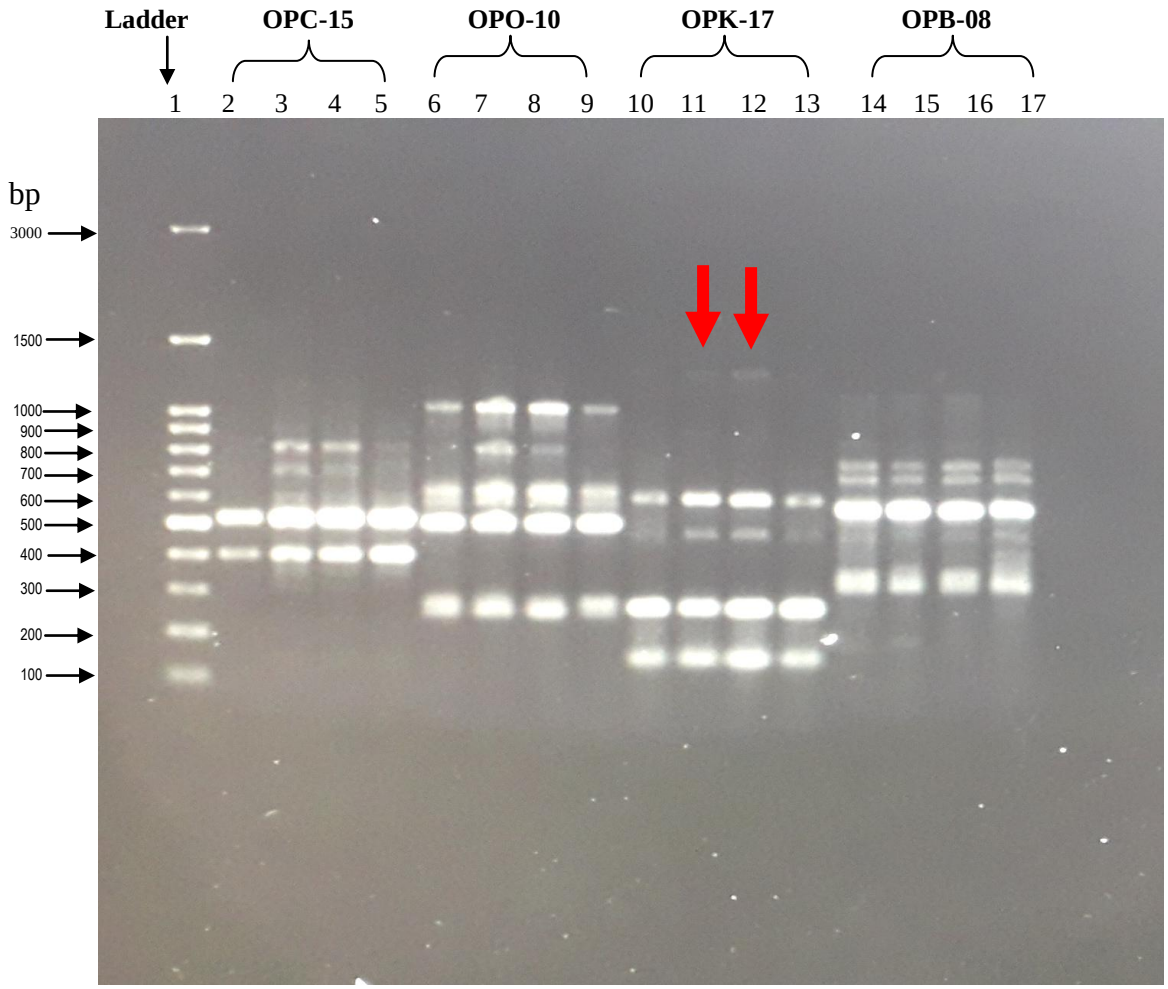

Ladder
OPC-15
OPO-10
OPK-17
OPB-08
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17
bp
3000
1500
1000
900
800
700
600
500
400
300
200
100

Fig 4.3 shows RAPD profile of four varieties using various primers (OPA 19, OPA 13, OPO 12, OPB 10). For primer OPA 19 (Lane 2 to 5), many DNA bands were seen. Maximum bands were present in Narendra Rashmi and bands were of 1500 bp, 1050 bp, 510 bp, 310 bp and 200 bp. In Narendra Shivani bands were of 1050 bp and 310 bp. In Narendra Prabha bands were of 1050 bp and 310 bp. Only one bands was present in Narendra Madhuri band was of 1050 bp. This band was specific as present in Narendra Madhuri and Narendra Rashmi had specific band of 1500 bp and 510 bp. Narendra Shivani had specific band of 310 bp.

For primer OPA 13 (Lane 6 to 9), many DNA bands were seen. Maximum bands were present in Narendra Rashmi which were of 200 bp, 350 bp, 550 bp, and 750 bp, In Narendra Shivani bands were 200 bp, 350 bp, 550 bp and 750 bp In Narendra Prabha bands were 200 bp, 350 bp, 550 bp and 750 bp. Two bands only were present in Narendra Madhuri bands were of 200 bp and 350 bp. For this primer there was only one band present in Narendra Madhuri which was of 350 bp.

For primer OPO 12 (Lane 10 to 13), also many DNA bands were seen. Maximum bands were present in Narendra Madhuri bands of 200 bp, 250 bp, 750 bp and 1000 bp. In Narendra Rashmi bands were 200 bp, 250 bp, 750 bp and 1000 bp. In Narendra Shivani bands were 200 bp, 250 bp, 750 bp and 1000 bp. Two bands were present in Narendra Prabha bands were of 200 bp and 1050 bp. Narendra Madhuri had a band that was 750 bp. Narendra Rashmi had a band that was 750 bp. Narendra Shivani had a band that was 750 bp.

For primer OPB 10 (Lane 14 to 17), many bands were also seen. Maximum bands were present in Narendra Madhuri of size 150 bp, 250 bp, 450 bp and 650 bp. In Narendra Rashmi bands were of 150 bp, 250 bp, 450 bp and 650 bp. In Narendra Shivani bands were of 150 bp, 250 bp, 450 bp and 650 bp. Three bands was present in Narendra Prabha which were of 250 bp, 450 bp and 650 bp.

There was no specific band corresponding long variety in above used four primer sets.

Fig 4.4 shows RAPD profile of four varieties using various primers (OPC 15, OPO 10, OPK 17, OPB 08). For primer OPC 15 (Lane 2 to 5), many bands amplified. Maximum bands were present in Narendra Rashmi and Narendra Shivani. In Narendra Rashmi bands were of 400 bp, 510 bp, 700 bp and 810 bp. In Narendra Shivani it was 400 bp, 510 bp, 700 bp and 810 bp. Two bands were present in Narendra Madhuri and Narendra Prabha bands were of 400 bp and 510 bp. Narendra Rashmi and Narendra Shivani had a specific band of 810 bp.

For primer OPO 10 (Lane 6 to 9), many bands were seen. Maximum bands were present in Narendra Rashmi bands of 200 bp, 510 bp, 610 bp, 810 bp and 1010 bp. In Narendra Shivani bands were 200 bp, 510 bp, 610 bp, 810 bp and 1010 bp. Minimum bands were present in Narendra Madhuri of 200 bp, 510 bp, 610 bp of 1010 bp. In Narendra Prabha bands were of 200 bp, 510 bp, 610 bp and 1010 bp. Specific band were present in Narendra Rashmi and Narendra Shivani of 810 bp.

For primer OPK 17 (Lane 10 to 14), many bands amplified in all varieties. Maximum bands were present in Narendra Rashmi and Narendra Shivani. In Narendra Rashmi bands were of 100 bp, 250 bp, 500 bp, 600 bp and 1200 bp. In Narendra Shivani bands were of 100 bp, 250 bp, 500 bp, 600 bp and 1200 bp. Minimum bands were present in Narendra Madhuri bands was 100 bp, 250 bp and 600 bp. In Narendra Prabha bands were of 150 bp, 250 bp and 600 bp. Specific band were present in Narendra Rashmi and Narendra Shivani which was 1200 bp.

For primer OPB 08 (14 to 17), many bands were also seen. Maximum bands were present in Narendra Madhuri bands of 300 bp, 650 bp, 750 bp and 850 bp. In Narendra Rashmi 300 bp, 650 bp, 750 bp and 850 bp. Minimum bands were present in Narendra Shivani and Narendra Prabha of 300 bp and 650 bp, respectively.

With OPK 17, long specific DNA band of 1200 bp was seen in Narendra Shivani and Narendra Rashmi. Band was more intense in Narendra Shivani then Narendra Rashmi.

4.5 Dendrogram generation using NTSYS-pc

Fig 4.5.1 shows dendrogram of all four varieties after NTSYS analysis. Bands of each varieties with each primer were scored giving 1 for DNA band present and 0 for no band of same size. NTSYS analysis showed their genetic coefficient as shown in figure Narendra Rashmi and Narendra Shivani falls in one group while Narendra Madhuri and Narendra Prabha in different group. This suggests that long variety are clustered together.

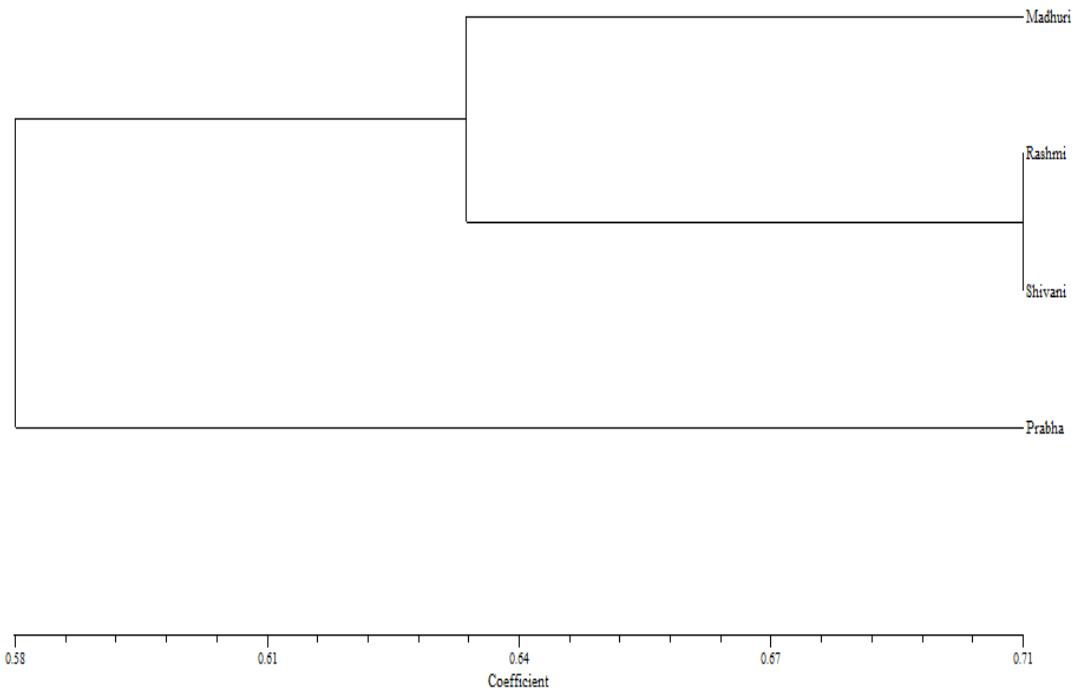
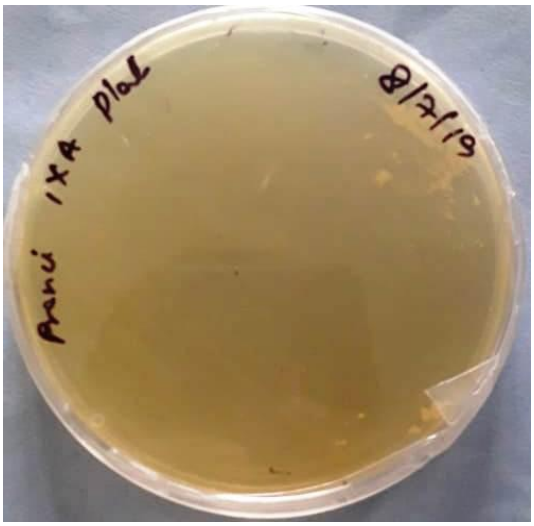


Fig. 4.5.1: Dendogram of *Lagenaria*

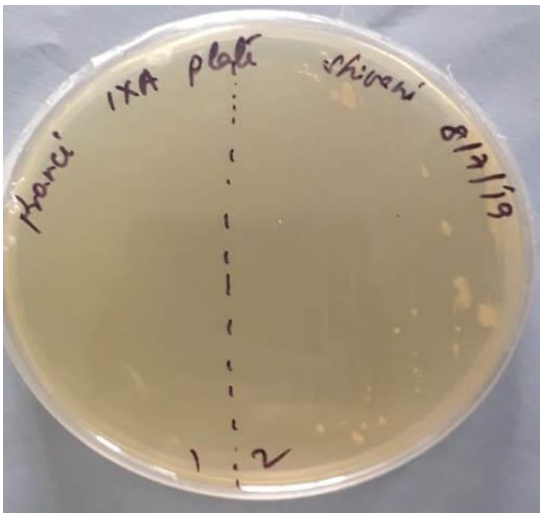
4.6 Cloning in long specific DNA in TA Cloning vector:

PCR product was cloned in TA vector. For this 1200 bp PCR product was first eluted from agrose gel as shown in Fig. 4.6.1.

Long specific DNA after elution from gel was transformed as described in method and material and was put in TA cloning vector. From blue and white colony, white colony was selected as recombinant.



Narendra Rashmi



Narendra Shivani

Chapter-V

DISCUSSION

Broad objective of this work was to find out why Narendra Shivani variety is too long (up to 2 to 8 feet). To address this objective approach we took as first do fingerprinting of long and round varieties and established their relationship. Following this differentiate between round and long varieties of bottle gourd and again explore what makes Shivani very long.

As per requirement first DNA was isolated from the all varieties. Teethed and no sharing definitely suggest that isolated DNA is intact in all varieties and can be used further molecular work.

DNA markers are abundant and essentially independent from environmental conditions (Keim *et al*., 1989), and several research group have been using this tool in breeding programs (Shoemaker *et al*., 1992; Young and Kelly, 1996; Young *et al*., 1998). Further RAPD profiles appear to be useful in discriminating cultivars and show promise as identification makers.

As shown in figure 3.2.2 and 3.2.3 out of 32 RAPD primers 8 primers shown good amplification. Unique polymorphic bands produced by these primer were able to identified the genotype. Cluster analysis based on RAPD marker grouped the genotype in three main clusters.

Analysis of RAPD variation revealed that the evolutionary history of bottle gourd is complex. Long varieties fall in one group but round

Variety Madhuri and Prabha were distant apart. Previous studies of bottle gourd also suggests fragment size variation of total genomic DNA using RAPD primers (Decker *et al.,* 2001)

It can be observed that brightness and position of DNA bands are not always exact. It could be explained by DNA molecular variation in different genotype and heterogeneity or heterozygosity.

The DNA fragment corresponding to RAPD maker OPK_{1200} was cloned and partially sequenced for making SCAR marker. The reliability of SCAR makers linked to a unique locus has been reported in some important crops (Naqvi and Chattoo., 1996; Barret *et al.,* 1998) as well as in grapevine (Lahogue *et al.,* 1998).

Further, the DNA fingerprints of *Lagenaria* developed would be of immense use identifying these genotype individually which would be useful in plant variation rights to safe guard the countries genetic resources.

Chapter-VI

SUMMARY AND CONCLUSION

DNA was isolated from 4 varieties of *Lagenaria* (2 long Narendra Shivani and Narendra Rashmi and 2 Round Narendra Madhuri and Narendra Prabha) and was done with 32 primers.

RAPD was done in 4 varieties of *Lagenaria* (2 long Narendra Shivani and Narendra Rashmi and 2 Round Narendra Madhuri and Narendra Prabha) and was done with 32 primers.

Out of 32 primers, 8 primers shown good amplification and dendrogram was generated using NTSYS analysis.

Relationship of *Lagenaria* was established as shown by dendrogram. Narendra Rashmi and Narendra Shivani (both long) falls in one group as shown in dendrogram.

1200 bp long variety specific DNA was identified with OPK 17 primer. 1200 bp band in Narendra Shivani was more intense in Narendra Shivani than Narendra Rashmi.

Longest nature of Narendra Shivani may be due to repeat copy of this DNA as amplican was more intense in Narendra Shivani then Narendra Rashmi though it needs confirmation.

Chapter-VII

BIBLIOGRAPHY

Achigan-Dako, E. G., Fagbemissi, R., Avohou, H. T., Vodouhe, R. S., Coulibaly, O. and Ahanchede, A. (2008) Importance and practices of Egusi crops (*Citrullus lanatus)* Matsum and Nakai, *Cucumeropsis mannii* Naudin and *Lagenaria siceraria* (Molina) Standl. cv. Aklamkpa in Sociolinguistic areas in Benin. *Biotechnol. Agron. Soc. Environ*.,12:393-403.

Achu, M., B., Fokou, E. Kansci, G. and Fots, M. (2013) Chemical evaluation of protein quality and phenolic compound levels of some Cucurbitaceae oilseeds from Cameroon. *African Journal of Biotechnology,* 12(7):735-743.

Azazy, S. G. A., Razek, A. B. A., Soliman K. A. and Ibrahim, S. A. (2011) Biochemical and molecular identification Bottle Gourd (*Lagenaria siceraria)* cultivar. *Egypt. J. Genet. Cytol.,* 40: 187-199.

Baranowska, M. K. and Cisowski W. (1995) Flavone *C*-glycosides from *Bryonia alba* and *B. dioica*. *Phytochemistry*, 39(3):727-729.

Barret, P., Delourme, R., Foisset, N. and Renard, M. (1998) Development of a SCAR (Sequence characterised amplified region) marker for molecular tagging of the dwarf BREIZH (Bzh) gene in *Brassica napus* L. *Theor. Appl. Genet.,* 97:828-833.

Beevy, Kuriachan (1996) Chromosome number of South India Cucurbitaceae and a note the cytological evolution in the family. *J Cytol Genet*, 31:65-71.

Behera, T. K., (2008) Amplified Fragment Length Polymorphism Analysis Provides Strategies for Improvement of Bitter Gourd (*Momordica charantia)* L. *Horti. Sciece*, 43:127-133.

Bhagyawant, S. S., (2010) RAPD-SCAR Markers: An interface tool for authentication of traits. *Journal of Bio sciences and Medicines,* 04:1-9.

Bradeen J.M., Staub J. E., Wye C., Antonise R., and Peleman J. (2001) Towards an expanded and integrated linkage map of cucumber (*Cucumis sativus* L.). *Genome* 44: 111–119.

Chaudhary, H. and Ram, H.H. (2000) SDS-PAGE in cucurbits. *Veg. Sci.,* 27: 35-38

Cheng, J. L., Li, J., Qiu, X. M. L. Wei, Yang, L. Q., and Fu, J. J. (2016) Development of novel SCAR marker for genetic characterization of *Lonicera japonica* from high GC-RAMPPCR and DNA cloning. *Genetic and Molecular Research,* 15(2):12.

Clarke, A. C., Burtenshaw, M. K., McLenachan, P. A., Erickson, D. L., and Penny D. (2006) Reconstructing the Origins and Dispersal of the Polynesian Bottle Gourd *(Lagenaria siceraria*). *Molecular Biology and Evolution*, 23(5):893-900.

Decker, W. D., Staub, J., Lopez, S. A. and Nakata, E. (2001) Diversity in landraces and cultivars of bottle gourd *(Lageraria siceraria*; Cucurbitaceae) as assessed by random amplified polymorphic DNA. *Genetic Resources and Crop Evolution,* 48:369-380.

Deena S.D., Ellert M.W., Chung M.S., and Stuab E.J. (2004) Discovery and genetic assessment of wild bottle gourd *Lagenaria Sicerania* (Mol.) Standley; Cucurbitaceae from Zimbabwe. *Economic Botany*, 58:501-508.

Devaiah, K., Balasubramani, S. P. and Venkatasubramanian, P. (2011) Development of Randomly amplified polymorphic DNA based SCAR maker for identification of *Ipomoea mauritiana* Jacq (Convolvulaceae). *Hindawi Publishing Coporation,* 2011:06.

Devi, T. P,. Prabhakaran, N., Kamil, D.,Borah, J. L. and Alemayehu, G. (2013) Development of SCAR marker for specific detection of *Aspergillus flaves. Academic Journals*, 09(9):783-790.

Dhanya, K., Syamkumar, S., Siju, S. and Sasikumar, B. (2011) Sequence characterized amplified region makers: Areleable tool for adulterant detection in turmeric powder. *Food Research International*, 673012:07.

Dje, Y., Tahi, C. G., Zoro Bi, A. I., Baudoin, J. P. and Bertin, P. (2010) Use of ISSR markers toassess genetic diversity of African edible seeded *Citrullus lanatus* landraces. *Sci. Horti,* 124:159-164.

Dje, Y., Tahi, G. C., Zoro, B., Malice, M., Baudoin, J. P and Bertin P. (2006) Optimization of ISSR marker for African edible-seeded Cucurbitaceae species genetic diversity analysis. *African Journal of biotechnology*, 5(2):83-87.

Dubey, K.R. and Ram, H.H. (2008) Characterization of advanced breeding lines and assessment of genetic diversity in bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] through SDS-PAGE. *Int. J. Plant Breeding,* 2: 85-86.

EL-Hammady, Khalifa, A. H. and Ramadan, B. R. (2001) Use of bottle gourd (*Lagenaria siceraria)* seeds in production of Tahina. *Egypt. J. Food Sci*, 29:1-11.

Enslin, P. R. (1994) Bitter principles of the cucurbitaceae. I. Observations on the chemistry of cucurbitacin. *Journal of the science of food and agriculture*. 5(9):410-416.

Ferrial, M., Pico, M. B. and Nuez, F. (2004) Genetic diversity of some accessions of Cucurbita maxima from Spain using RAPD and SBAP markers. *Genetic Resources and Crop Evolution,* 50:230-238.

Filho, S. M., Sedigama, C. S., Moreira, M. A. and Gancalves, D. B. E. (2002) RAPD and SCAR markers linked to resistance to frogeye leaf spot in soyabean. *Genetic and Molecular Biology,* 25:317-321.

Garcia, J., Oliver, M., Paniagua, H. G. and Vicente, M.C. D. (2000) Comparing AFLP, RAPD and RFLP markers for measuring genetic diversity in melon. 101:Issue 5–6.

Ghosh, P., Shylaja, R.M. and Nazeem, A.P. (2014) RAPD Marker based DNA Fingerprinting in released varieties and selected superior Somaclones of Ginger (*Zingiber officinale* Rosc.). *International Journal of Plant, Animal and Environmental Sciences*, 4 (2) : *2231-4490.*

Hassan, L. G., Sani, N. A., Dangoggo, S. M. and Ladan, M. J. (2008) Nutritional value of bottle gourd (*Lagenaria siceraria*) seeds. *Global Journal of Pure Applied Science*, 14(3):301-306.

Hegazy, E. M. and Kinway, O. S. (2011) Characteristics of Pumpkin and bottle gourd in Egypt and Potentially their contamination by Mycotoxin and Mycotoxigenic Fungi. *Journal of American Science,* 7(9):615-622.

Heidelberg (2003) Assessment of genetic relationships in *Cucurbita pepo* (Cucurbitaceae) using DNA markers. *Theoretical and applied genetics*, 106: 971-978.

Heikal, Hadia, A., Razzak, H.S. and Hafez, E.E. (2008) Assessment of genetic relationship among and within cucurbita species using RAPD and ISSR markers. *J. Applied Sci. Res,* 4: 515-525.

Heiser, C. B. (1979) The gourd book. University of Oklahoma,.235p.

Heiser, J. (1989) The evolution of plant exploitation: Harris, Dr. Hillman, G. C., Foraging and Farming. Unwin Hyrnan, London, 471-480.

Hoque, S. and Rabbani, M.G. (2009) Assessment of genetic relationship among landraces of Bangladesh Ridge Gourd (*Luffa acutangula* Roxb.) using RAPD markers. *J. Scientific Res.,* 1: 615-623.

Horticulture Statistics at a glance. 257:1-481.

Huanwenmeng, Shuxiachen , Zhihuicheng, Chai, D. and Yali, li. (2012). Srap markers for fruit shape in cucumber. *Yangling Shaanxi,* 44: 1381-1384.

Ilknur, S., Sar N., Kacar, Y.A. and Mendi, N.Y. (2010) The genetic characterization of Turkish watermelon (*Citrullus lanatus*) accession using RAPD markers. *Genetic Resources and Crop Evolution,* 57: 763-771.

Islam, M. N., Molla, R., Rohman, M., Hasanuzzaman, M., Islam, S. M. N. and Rahman, L.(2012) DNA Fingerprinting and Genotyping of Cotton Varieties Using SSR Markers. *Not Bot Horti Agrobo,* 40(2):261-265.

Jack, T.J. (1986) Cucurbit Seed Protein and Oil in plant Protein: Applications, Biological Effects and Chemistry. Ed. Robert L. Ory. U.S. Department of Agriculture/ American Chemical Society, Washington D.C.

James, M. and Stephens, M.J. (2015) Gourd Bottle- *Lagenaria siceraria (*Mol.) Standl. *IFAS Extension, University of Florida*, HS, 4: 602.

James, P. and Varsa, N. (2002) Development of SCAR marker for DNA fingerprinting and germplasm analysis of American Cranberry. *J. Amer. Soc. Hort. Sci.,* 127(4):677-684.

Jarial, K., Dogra, B., Mandradia, R. K., Kumar, S., Sharma, D. and Gupta, A. K.(2011) Investigations on a new bacterial disease of bottle gourd in sub-tropical zone of Himachal Pradesh. *Pl. Dis. Res*, 20 (1):68-75.

Jekishandas,M., Patel, H., R., Mohanlal, and Parshottambhai, S. K. (2017) Validation of molecular Markers linked to Fusarium Wilt resistance in Chickpea genotypes. *International Journal of Pure and Applied Bioscience,* 05:254-261.

Katare, C., Saxena, S. and Agrawal, S. (2014) Lipid-Lowering and Antioxidant Functions of Bottle Gourd (*Lagenaria siceraria*) Extract in Human *Dyslipidemia*. 03:345-351.

Kaur, G. and Aggarwal, P. (2014) Storage Studies on Bottle Gourd Juice Preserved with Different Chemical Additives. *Indian Journal of Applied Research*, 4 (2):2249-2255.

Keim, P., Shoemaker, R. C. and Palmer, R. G. (1989) Restriction fragment length polymorphism diversity in soyabean. *Theor Appl Genet,* 77:786-792.

Kesawat, M. S. and Das, B.K. (2009) Molecular makers: Application in crop improvement. J. crop Sci. Biotech. 12(4):172-178.

Kistler, L., Montenegro, A., Smith, B. D., Gifford, J. A., Green, R. E., Newsom L.A. and Shapiro, B. (2014) Transoceanic drift and the domestication of African bottle gourds in the Americas. *Natl Acad Sci U S A,*111(8): 2937–2941.

Kobayashi S.I. and Yoshida T (2007) Classification of bottle gourd (*Langenaria siceraria*) by RAPD analysis. *Japnese J. Crop Sci,* 76:93-99.

Koshan X. (2007) Gourd germplasm resources RAPD analysis of the genetic diversity of *L. siceraria. Molecular Plant Breeding*, 22:34-40.

L. Kiran, J. Arvind and R. Aradhita (2015) A Study on Quality Changes in Stored Bottle Gourd (*Lagenaria siceraria*) Juice. *Journal of food science and technology*, 4(1):21-27.

Lahogue, F., This, P. and Bouquet, A. (1998) Identification of co-dominant SCAR marker linked to the seedlessness character in grapevine. *Theor. Appl, Genet.* 97:950-959.

Lebeda, A. and Dolezal, K. (1995) Isozyme studies in cucurbits. *Zeitschriftfur Pflanzenkrankheitenuund Pflanzenschutz.* 102: 467-471.

Lee, J. M. (1994) Cultivation of grafted vegetable, Current status, grafting methods and benefits. *Hort. Science*, 29:235-239.

Levi, A. and Thomas, C. E. (2004) ISSR and AFLP markers differ among America Watermelon cultivars with limited genetic diversity. *J. Amer. Soc. Hort. Sci,* 129(4):553-558.

Lynch, M. and Miligan, M. (1994) Analysis of population genetic structure with RAPD markers. *Mol. Ecol*, 3(2):91-99.

Mahendra, T. K., Alice, B., Dahryn, T., Gopal, N., Mayank, G. and J. Snehasis (2015) Morphological and Molecular Analysis Using RAPD in Biofield Treated Sponge and Bitter Gourd. *American journal of agriculture and forestry*, 03:264-270.

Mahendra, T.K. (2008) DNA finger printing by RAPD analysis in bottle gourd [*Lagenaria siceraria* (Mol.) Standl.]. *TAG*, 101: 1432-2242.

Maisuria, Jekishandas, H., Patel, Mohanlal, R. and Suthar, Parshottambhai, K (2017) Validation of Molecular Markers Linked to Fusarium Wilt Resistance in Chickpea Genotypes. *Int. J. Pure App. Biosci.* 5 (1): 254-260.

Mashilo, J., Shimelis, H., Odindo, A. and Amelework, B. (2016) Genetic Diversity of South African Bottle Gourd [*Lagenaria siceraria* (Molina) Standl.] Landraces Revealed by Simple Sequence Repeat Marker. *Horti sciece,* 51(2):120–126.

Milind, P. and Satbir, K. (2011) Is bottle gourd a natural Gourd? International Research Journal of Pharmacy, 2(6):13-17.

Minocha, S. (2015) An overview on *Lagenaria siceraria* (Bottle gourd). *Journal of Biomedical and Pharmaceutical Research,* 4(3): 04-10.

Mirlosawa, K. B. and Cisowski, W. (1995) Isolation and identification of C-glycosides flavone from *Lagenaria siceraria. L. L. D Res.* 52:137-9.

Mladenovic, E., Berenji, J., Ognjanov, V., Ljubojevic, M. and Cukanovic, J.(2012) Genetic variability of bottle gourd *Lagenaria siceraria* (Mol.) Standley and its morphological characterization by multivariate analysis. *Arch. Biol. Sci.,* 64 (2):573-583.

Murray, M. G. and Thompson, W. F. (1980) Rapid isolation of high molecular weight plant DNA. *Nucleic Acids Res*, 8(19): 4321–4325.

Nagaraja, N. R., Singh, A., Pallavi, J. K., sharama, J. B., Singh, G. P., Jain, N. and Prabhu, K. V. (2013) Validation of molecular markers linked to the stem rust resistance genes effective in India. *Indian J. Genet,*73(3):314-319.

Nagendran, K., Karthika, C., Parthasarathy, S., Kumar, M. S. and Karthikeyan, G (2016) Molecular evidence for the occurrence of Tomato leaf curl New Delhi virus on bottle gourd in Tamil Nadu, India. *Vegetable Science*, 43 (1):73-77

Najafiniya, M., and Sharma, P., (2011) Specific PCR based maker foe detection of pathogenic groups of *Fusarium oxysporium* f. sp. *Cucumerium* in India. *Journal of Genetic Engineering and Biotechnology,* 09:29-34.

Naqvi, N. I. and Chattoo, B. B. (1996) Development of a sequence characterised amplified region (SCAR) based indirect selection method for a dominant blast resistance gene in rice. *Genome* 39:26-30.

Nasul, I. M., Molla, M. R., R. Md. M., Hasanuzzaman, M., Naimul, I. S. M. and Lutfur, R. (2012) DNA fingerprinting and genotyping of cotton varieties using SSR markers. *Not Bot Horti Agrobo*, 40:261-265.

Paris, H.S.; Portnoy, V.; Mozes- Daube N.; Tzuri, G.; Katzir, N. and Yonash, N. (2010) AFLP, ISSR and SSR polymorphism are in according with botanical and cultivated plant taxonomies of the highly polymorphic. *Cucurbita pep.* 8:110-114.

Piccirilli, A., J. Smadja, P. Msika, I. Grondi and N. Piccardi (2007) Use of an oil of the gourd family for inhibiting 5 alpha reductage activity. *United state Patent Washington USA,* 1-9.

Prajapati R. P., Kalari, M. V., Karkare, V. P., Parmar, S. and Sheth, N.R. (2011) Effect of methanolic extract of *Lagenaria siceraria* (Molina) Standley fruits on marble-burying behavior in mice: Implications for obsessive-compulsive disorder. 03:62-66.

Prajapati, R. P., Kalariya, M., Parmar, S. K. and Sheth, R. (2010) Phytochemical and pharmacological review of *Lagenaria sicereria. J Ayurveda Integr Med,*1(4): 266–272.

Prashar, Y., Gill, N. S. and Perween, A. (2014) An updated review on medicinal properties of *Lagenaria siceraria. Int. J. Universal Pharm. Bio Sci,* 3:362-376.

Rahman, A. S. H. (2003) Bottle gourd A vegetable for good health. *Natural Product Radiance*, 2(5):249-257.

Rai, R., Das, B. K. and Bhagwat, S. G. (2017) Development and validation of scar maker for stem rust resistance gene sr 26 in wheat (*Triticum aestivum*). *Biological System Open Access*, 06:04.

Ram, H.H., Sharma, K. and Jaiswal, H.R. (2006) Molecular characterization of promising genotype in bottle guard including a novel segmented leaf type through RAPD. *Veg Sc,* 33(1): 1-4.

Rambabu, E., Mandal, A. R. azra, P. H. Senapati, B. K. and Thapa, U. (2017) Morphological characterization and genetic variability studies in Bottle gourd [*Lagenaria sicecaris*]. *International Journal of Current Microbiology and Applied Science,* 06:3585-3592.

Rathod, V., Narasegowda, C, N., Papanna, N. and Simon, L. (2008) Evaluation of genetic diversity and Genome fingerprinting of Bitter gourd genotype (*Momordica charantia* L.) Morphological and RAPD Markers. *International Journal of Plant Breeding*, 2(2):79-84.

Riaz, S., Sadia, B., Awan, F. S., Khan, I. A. and Sadaqat, H. A. (2012) Development of species specific sequence characterized amplified region marker for roses. *Genetic and Molecular research,* 11(1):440-447.

Rugienius, R., Siksnianas, T., Stanys, V., Gelvonauskiene, D. and Bendokas, V. (2006) Use of RAPD and SCAR makers for

identification of strawberry genotypes caring red stele (*Phytophtora fragariae*). *Agronomy Research,* 04:335-339.

Sadia, A. (2007) Genetic Diversity in rose based on RAPD analysis. M.Sc. Dissertation thesis, University of Agriculture, Faisalabad, Pakistan.

Said, P.P., Sharma, N., Naik, B. and Pradhan, R. C. (2014) Effect of Pressure, Temperature and flow rate on Supercritical Carbon Dioxide Extraction of Bottle Gourd seed oil. *International Journal of food and Nutritional Sciences,* 3(3):14-17.

Sairkar, P. K., Sharma, A., and Shukla, N. P. (2016) SCAR Marker for identification and discrimination of *Commiphora wightii* and *C. myrrna*. *Molecular Biology Internatinal,* ArticleID 1482796,10.

Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning: A Laboratory Manual, (2nd ed.). Cold Spring Harbor: Cold Spring Harbor Laboratory Press.

Semsang, N., Semsang, R., and Phanchisri, B. (2013) Development of a SCAR marker for discrimination of a Thai Jasmine Rice (*Oryza sativa*) mutant, BKOS6, and association with purple colour trait in Thai Jasmine Rice related verities. *American Journal of Plant Science,* 04:1774-1783.

Shan, G.; Xiang, X.D.; Ying, B.N.L. and Kaiqin, Z. (2008) Genetic diversity analysis of 38 bottle gourd [*Lagenaria siceraria* (Mol.) Stand]. Accession by ISSR. *J. Plant Genetic Resources,* 4:396-400.

Shivakumar, M., Gireesh, C., and Ramesh, S. V. (2014) DNA finger Printing – A Tool for identification of cultivars. *Pop. Kheti,* 2(2):160-162.

Shoemaker, R. C., Guffi, R. D., Lorenzen, L. L. and Specht, J. E. (1992) Molecular genetic mapping of soyabean: map utilization. *Crop Sci,* 32:1091-1098.

Sikdar, B., Bhattacharya, M., Mukherjee, A., Benerjee, A., Ghose, E., Ghose, B. and Roy, S.C. (2010) Genetic diversity in important members of cucubitaceae using isozyme, RAPD and ISSR markers. *Biologia Plantarum,* 54: 135-140.

Singh, D. P., Mishra, U. C., Prakash, H. G. and Mishra, O. (2012) Role of organic farming on yield and economics of bottle gourd after vegetable pea. *Int. J. Agric. Sci.,* 8:165-167.

Singh, R.P., Jain, P.K., Gontia, A. S. and Verma, A. K. (2017) Physiological Evaluation of different Genotypes and their F1 Progenies in Bottle Gourd (*Lagenaria siceraria* Mol. Standl. *International Journal of Chemical Studies*, 5(3): 74-76.

Singh, S. P., Maurya, I. B. and Singh, N. K. (1996) Occurrence of andromonoecous form in Bottle gourd (*lagenaria siceraria*) exhibiting monogenic recessive inheritance. *Current Science*,70:458-459.

Sirohi, P. S. and Sivakami, N. (1991) Genetic diversity in cucurbits. *Indian Horticulture,* 36:45-46.

Sivaraj, N. and Pandravada, S.R. (2005) Morphological Diversity for Fruit Characters in Bottle Gourd Germplasm from Tribal Pockets of Telangana Region of Andhra Pradesh, India. *Asian Agri-History Foundation*, 9:305-310.

Staub J.E.; Robbins, M. D.; Chung, S. and Lopez-Sese A.I. (2004) Molecular methodologies for improved genetic diversity assessment in cucumber and melon. *Acta Horti,* 637, 41-47.

Staub, J., E. and serquen, F., C (2001) Towards an integrated linkage map of cucumber: Map merging**.** *Acta horticulturae,* 510:357-366.

Stephens, M.J. (2015) Gourd, Bottle-*Lagenaria siceraria* (Mol.) Standl. U.S. Department of Agriculture, *UF/IFAS Extension Service,University of Florida, HS*, 4: 602.

Sukhlecha, A.,(2012). Bitter bottle gourd *(Lagenaria siceraria)*: Healer or killer? International journal of nutrition, Pharmacology, Neurological Diseases. 2(3):276-277

Tan, Y. C., Lai, J. S. , Adhikari, K. R., Shakya, S. M., Shukla, A. K. and K. R. Sharma (2009) Efficacy of mulching, irrigation and nitrogen applications on bottle gourd and okra for yield improvement and crop diversification. *Irrigation and drainage system,* 23(1):25-41.

Tewari, D. (1997). SDS-PAGE in cucurbits. *Agric. Rev.,* 29: 97-99.

Trivedi, M. K., Branton, A., Trivedi, D., Nayak, G., Gangwar, M. and Jana, S.(2015) Evaluation of Vegetative Growth Parameters in Biofield Treated Bottle Gourd (*Lagenaria siceraria*) and Okra

(*Abelmoschus esculentus). International Journal of Nutrition and Food Sciences,* 4(6):688-694.

Walter, D. D., Staub, J., Sese, A. L. and Nakata E. (2001) The origin and genetic affinities of wild populations of melon (*Cucumis melo,* Cucurbitaceae) in North America. *Genetic Resources and Evolution,* 48: 369-380.

Walters, D. S. D., Staub, J. E., Chung, S. M., Nakata, E. and Quemada, H. D. (2002) Diversity in free living polulations of *Cucurbita pepo* (Cucuritaceae) assessed by Random Amplified Polymorphic DNA. *Systematic Botony*, 27(1):19-28.

Wang, L.; XiaoHua, W.; Wang, B. and Guoling X. P. L. (2011) SCAR marker linked to resistance gene of mildew in bottle gourd (*Lagenaria siceraria* (Molina) Standl). *J. of Zhejiang University (Agriculture and Life Science)*, 37:119-124.

Wang, S. H., Y, Li, Z. Q. Li, L. Chang and Li, L. (2015) Identification of an SCAR maker related to female phenotype in *Idesia polycorpa* Maxim. *Genetic and Molecular Research*, 14:2015-2022.

Whittaker, R. H. and Feeny, P. P. (1971) Allelochemic: Chemical interaction between Species, Science. *American Journal of Plant Science,* 171:757-770.

Willis, A. T. Smith, J. A. and O'Connor, J. J. (1966) Properties of some epidemic strain of *Staphylococcus aureus*. *The journal of Pathology and Bacteriology.* 92(2).

Wosu, E. and Ndukwu, B. C. (2007) Phytogeography and biodynamic assessement of species of cucurbits in the niger delta area of Nigeria. *Global Journal of Pure Applied Science,* 14(1):59-65.

WU, Xinyi, XU, Pei, WU, Xiaohua, WANG, Baogen, LU, Zhongfu and LI, Guojing (2017) Development of Insertion and Deletion Markers for Bottle Gourd Based on Restriction Site-associated DNA Sequencing Data. *Horticultural Plant Journal,* 03:13-16.

Xiang, X. D., Ying, L. B. and Kai-qin, Z. (2008) Genetic Diversity Analysis of 38 Bottle gourd (*Lagenaria siceraria)* accessions by ISSR. *Journal of Plant Genetic Resources.* 5(1):12-18.

Xiang, X.D., Ying, B.N., Qing, F.R. and Zhang, L.Y. (2007) RAPD genetic diversity of bitter gourd germplasm and ISSR analysis. *J. Plant Genetic Resources,* 6: 450-454.

Xu, P., Wu, X., Luo, J., Wang, B., Liu, Y., Ehlers, D, J., Wang S., , Lu, Z. and Li, G. (2011) Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding Partial sequencing of the bottle gourd genome reveals markers useful for phylogenetic analysis and breeding. *BMC Genomics*, 12:467-45.

Yang, L., Fu S., Khan M. A., Zeng, W., and Fu, J. (2013) Molecular cloning and development of RAPD-SCAR markers for *Dimocarpus longan* variety authentication. *Springer Plus Journal,* 02:08.

Yasuyoki, M., Maundu, P., Kawase, M., Fujimaki, H. and Morishim H. (2005) RAPD polymorphism of the white flowered gourd [*L. Siceraria*] landraces its wild relatives in Kenya. *Genetic Resources and crop Evolution*, 53:963-974.

Yetisir, H. and Sari, N. (2003) Effect of different rootstock on plant growth, yield and quality of watermelon. *Australian Journal of Experiment Agriculture*, 43:1239-1274.

Young, R. and Kelly, J. D. (1996) RAPD markers flanking the are gene for anthracnose resistance in common bean. *J Amer Soc Sci* 121:37-41.

Young, R., Melotto, M, Nodari, R. O. and Kelly, J. D. (1998) Marker assisted dissection of the oligogenic anthracnose resistance in the common bean cultivar G2333. *Theor Appl Genet* 96:87-94.

DEPARTMENT OF PLANT MOLECULAR BIOLOGY AND GENETIC ENGINEERING
N.D. University of Agriculture & Technology, Kumarganj, Ayodhya-224 229 (U.P.) India

Major Advisor and Chairman
Dr. N.A. Khan
Associate Professor
Department of PMB & GE

Name of Student
Pranci Tiwari
I.D. No.: A 10048/17

Title: "Making Sequence Characterized Amplified Region marker for *Lagenaria* (Bottle Gourd)''

ABSTRACT

Bottle gourd or calabash [*Lagenaria siceraria* (Mol.) Standl.] (2n = 2x = 22), also known as opo squash or long melon, is diploid belonging to the genus *Lagenaria* of the Cucurbitaceae family with a genome size of ~334 Mb. Bottle gourd comes under in different sizes some are long, some are round and some are dumbbell shaped. Out of these Narendra Shivani is very long variety (up to 8 feet). Our interest was to know why Shivani variety is so long? So first effort was made the differentiate between round and long variety of Bottle gourd with 32 RAPD markers. Rashmi and Shivani (both long) falls in one group as shown in dendrogram. Long variety specific DNA was identified with OPK 17 of 1200 bp. Band in Narendra Shivani was more intense in Narendra Shivani than Narendra Rashmi and may be due to repeat copy though it needs confirmation.

(N.A. Khan) **(Pranci Tiwari)**