GENETIC DIVERSITY ANALYSIS IN GROUNDNUT (*Arachis hypogaea* L.) BASED ON MOLECULAR MARKERS AND BIOCHEMICAL PARAMETERS

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PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY

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CERTIFICATE-II

Date: 14/09/2022

This is to certify that the thesis entitled "**GENETIC DIVERSITY ANALYSIS IN GROUNDNUT (*Arachis hypogaea* L.) BASED ON MOLECULAR MARKERS AND BIOCHEMICAL PARAMETERS**" submitted by **Miss JIVANI JANKIBEN MANISHKUMAR (Reg. No. 2010120038)** to Junagadh Agricultural University, Junagadh in partial fulfilment of the requirements for award of the degree of **MASTER OF SCIENCE (AGRICULTURE)** in the subject of **PLANT MOLECULAR BIOLOGY AND BIOTECHNOLOGY** after recommendation by the external examiners were defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination was satisfactory. We, therefore, forward with recommendation.

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C E R T I F I C A T E - I

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Place: Junagadh
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GENETIC DIVERSITY ANALYSIS IN GROUNDNUT (*Arachis hypogaea* L.) BASED ON MOLECULAR MARKERS AND BIOCHEMICAL PARAMETERS

ABSTRACT

The present investigation on "Genetic Diversity Analysis in Groundnut (*Arachis hypogaea* L.) Based on Molecular Markers and Biochemical Parameters" was conducted at Department of Biotechnology, Junagadh Agricultural University, Junagadh with objectives to analyze different groundnut varieties for biochemical and molecular diversity using various PCR based molecular markers *viz.*, Random Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSRs) and Simple Sequence Repeats (SSRs) as well as to find out the phylogenic relationship among different groundnut varieties.

Eighteen groundnut varieties were selected for the biochemical and molecular study. Among the proximate parameters, the moisture content ranged between 5.59 to 9.12% in KDG-123 and GG-41, respectively. The highest value of oil content observed in the variety GJG-32 with 53.56%, while the lowest value of oil content was recorded in the variety Kaushal with 46.16%. Total carbohydrate content was maximum 22.96% in the variety GG-HPS-2, while the minimum value was 14.49% with variety GJG-32. The lowest value for total protein and true protein were observed in the variety GG-21 and JL-501 with 19.24% and 17.40%, respectively while, the highest value of total protein and true protein content with 24.84% and 20.98% in the variety KDG-123, respectively. Total soluble sugar content was maximum in the variety GG-HPS-2 with 11.95% and the minimum was in GJG-32 with 7.38%. The lowest reducing sugar content 1.67% was observed in the variety GJG-22 and the highest 3.05% was in the variety TG-37A. Maximum non reducing sugar content was recorded in the GG-HPS-

2 variety with 9.56%, while the minimum was in the variety GJG-32 with 4.87%. The lowest ash content 1.64% was observed in the variety GG-41 and the highest 3.27% was in the variety GJG-9.

Total 21 RAPD primers generated 118 bands in which all 98 bands were polymorphic having 91 shared and 7 unique bands with an average of 4.7 bands and 81.03% polymorphism per primer. The RAPD primers augmented fragment size ranged from 136 bp in primer OPQ-14 to 2680 bp in primer OPD-05. The 13 ISSR primers engendered 51 bands in which all 43 bands were polymorphic with 40 shared and 3 unique bands and had 72.12% polymorphism with an average of 3.30 bands per primer. The amplified fragments were in the range of 128 bp in UBC-805 to 1629 bp in UBC-806. Total 18 SSR primers were used which generated the total 37 fragments in which all 25 bands were polymorphic with 22 shared and 3 unique bands and had 53.70% polymorphism with an average of 1.39 bands per primer. Size of SSR primer amplified fragments were in the range of 107 bp in JAUGP-197 to 957 bp in JAUGP-175. An average polymorphism information content (PIC) value for RAPD primer, ISSR primer and SSR primer were 0.78, 0.63 and 0.27, respectively. Primer index for RAPD, ISSR and SSR were 4.64, 2.79 and 0.94, respectively.

The similarity coefficient of clusters analysis was ranged from 54 to 91% for RAPD, 33 to 92% for ISSR and 56 to 94% for SSR. Dendrogram construction of all these molecular markers showed that in RAPD, GG-HPS-2 variety of groundnut was the most diversified variety observed having alone separate position in cluster. In ISSR marker, TG-37A variety was found to be a most diverse in all the varieties. In SSR marker, KDG-128 variety was the most diversified. In pooled data, TG-37A and JL-501 were found as the most diversified varieties. The pooled analysis study of RAPD, ISSR and SSR generated clustering pattern which was found to be similar as ISSR clustering pattern.

The data generated from the present study may be useful for the identification of the genetic diversity among groundnut varieties which could be further utilized for molecular breeding and marker assisted selection in crop improvement programs.

Key words: Groundnut, Genetic diversity, Molecular markers, Biochemical parameters.

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Date : 05/07/2022 **(Jivani Jankiben M.)**

CHAPTER - I

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is an economically vital oilseed crop with high nutrition value of oils, proteins, vitamins and calories. Groundnut (*Arachis hypogaea* L.) is the plant of the Leguminosae family. Earthnuts, peanuts, gobber peas, monkey nuts, pygmy nuts, manila nuts, ground bean, and pignuts are some of the local names for groundnuts (Bhanu and Sadagopan, 2013).

The botanical name of groundnut (*Arachis hypogaea* L.) is derived from two Greek words, *Arachis* means a legume and *hypogaea* means below ground, referring to the formation of pods in the soil. Groundnut is also called as the "King of Oilseeds" or "Wonder nut" and "Poor man's cashewnut". Groundnut (*Arachis hypogaea*) is divided into two subspecies, *viz.*, ssp. *hypogaea* and ssp. *fastigiata* based on variation in morphology (Kalyani and Sasidharan, 2021). Peanut or groundnut (*Arachis hypogaea* L.) is a crucial crop for oil and protein source. It is native to South America but is now cultivated in over 80 countries between 40° N and 40° S in tropical and heat temperate areas of the world. Cultivated peanut has four botanical types, hypogaea, hirsuta, vulgaris and fastigiata (Yi *et al*., 2011).

Groundnut is a self-pollinated having chromosome number (2n=40) grown in tropical and sub-tropical parts of the world. Groundnut is believed to be the native of Brazil. It was introduced in India during the first half of the sixteenth century from one of the conciliatory islands of China, where it was introduced prior from either Central America or South America. India having second position in groundnut production after China. Groundnut is the largest oilseed producer crop in India in terms of production. Groundnut is one of the most important cash crops of our country (Aditya and Vikas, 2021). Groundnut is the 6th maximum crucial oilseed crop withinside the global and it is grown in 100 countries of the world both in the tropical and temperate zones. The main groundnut generating nations are China, India, USA, Indonesia and Senegal. In Asia greater than 28 nations domesticate groundnut and the predominant manufacturers are India, China, Indonesia, Myanmar and Vietnam. The USA is the most important consumer of groundnut in the world followed by Africa. Peanuts are normally used in the form of various food items such as roasted, ground or paste, peanut oil, boiled or

raw, the most commonly utilized form is the roasted peanut followed by peanut paste. In developing countries like India peanut seeds are specially used for groundnut oil extraction and its by-product is utilized as feed for animal husbandry (Bhanu and Sadagopan, 2013).

In India, groundnut is cultivated over an area of 47.308 lakh hectares with production of 67.272 lakh tonnes with a median yield of 1422 kg/ha within the year 2018-19. The most important groundnut growing states are Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, Maharashtra and Rajasthan. Gujarat is the largest producer of groundnuts in India. In Gujarat, groundnut is cultivated over an area of 15.942 lakh hectares with production of 22.028 lakh tonnes with an average yield of 1382 kg/ha in the year 2018-19 (Pal *et al*., 2021). Groundnut accounts around 25% of the entire oilseed production of the country. Annual production of Indian peanuts and Indian peanuts oil are around 5-8 million tonnes and 1.5 million, respectively. Peanuts Production is highly vulnerable to rainfall deviations and display huge fluctuation between years (Aditya and Vikas, 2021).

The genus Arachis contains 80 species, and maximum of them are diploid ($2n = 2x = 20$) with only two allotetraploids. The cultivated groundnut is allotetraploid (AABB, $2n = 4x = 40$), which is assumed to be the result of hybridization between two wild species, *Arachis duranensis* (AA-genome, $2n = 2x = 20$) named as “A-genome ancestor” and *Arachis ipaensis* (BB-genome, $2n = 2x = 20$) named as “B-genome ancestor” and subsequent chromosome doubling (Desmae *et al*., 2019).

Groundnut is classified based on growth habit as 1. Spreading type (Virginia Runner-VR) in which branches are spreading, main shoot may erect or bent, 2. Semi-spreading (Virginia Bunch-VB) type in which main shoot always erect and 3. Bunch type (Spanish Bunch-SB) in which branches makes acute angle with the erect main shoot.

Nutritional value of groundnut

Groundnut is plentiful in protein, healthy fats and dietary fibre. Groundnut is wealthy in calcium, potassium, phosphorus and B vitamins which gives you with a number of health benefits. Groundnut is an extraordinary combo of healthy fats, protein and fibre that curbs your appetite, lowers the threat of heart disease and controls blood glucose levels. Groundnut nutrition value per 100 grams as calories 567 kcal, total fat

49 g, saturated fat 7 g, polyunsaturated fat 16 g, monounsaturated fat 24 g, protein 26 g, total carbohydrate 16 g, dietary fiber 9 g, sugar 4 g, sodium 18 mg, potassium 705 mg, calcium 134 mg, iron 6.7 mg, magnesium 245 mg, phosphorus 549 mg, vitamin B1 0.9 mg, vitamin B2 0.2 mg, niacin 17.6 mg, vitamin B6 0.5 mg and folate 350 mcg (Anon., 2020).

In India, approximately 81% of the entire groundnut produced is used for oil extraction, 12% for seed, 6% for direct intake and 1% for export trade. Groundnut oil includes of triglycerides (96.1-96.4%), phospholipids (2.4-2.9%), sterols (0.69-0.80%), free fatty acids (0.1-0.4%) and glycolipids (0.10-0.14%). Eight fatty acids can be routinely detected in peanut seeds; however, two main fatty acids, oleic acid (C18:1, D9) and linoleic acid (C18:2, D9, D12), account for about 80% of the fatty acid composition. Groundnut oil contain major fatty acids are palmitic acid (8-11%), oleic acid (36-52%) and linoleic acid (24-43%). The biochemical difference among these two fatty acids is that linoleic acid contains one more double bond at the Δ12 position of the hydrocarbon chain than oleic acid. Fatty acid desaturase (FAD) enzyme allows the conversion of oleic acid to linoleic acid by adding double bond to oleic acid. This enzyme is coded by two homologous genes (ahFAD2A and ahFAD2B) positioned on A and B sub-genomes of groundnut. Consuming oils with excessive levels of oleic acid is useful to human health because it reduces low-density lipoproteins, maintain high-density lipoprotein, slow down atherosclerosis and reversing the inhibitory effect of insulin production. As a result, groundnut oil with a highest percentage of oleic acid is preferred and the quality of oil can be determined by the oleic acid and linoleic acid (O/L) ratio (Tinee *et al*., 2021).

Health benefits of groundnut

Peanut oil is extracted using a variety of methods and is primarily consumed on the Asian subcontinent, particularly in India. The vast majority of the world's peanut crop is used for oil production. Peanut lipid profile contains around 50% monounsaturated fatty acids (MUFAs), 33% paraformaldehyde (PFAs) and 14% saturated fatty acids, according to the American Peanut Council, which is a heart-friendly combination of fatty acids. When compared to low-fat diets, peanut products (raw, butter and oil) are more beneficial to heart health. The high monounsaturated fat peanut diets reduced total body cholesterol by 11% and bad LDL cholesterol by 14%, while triglycerides were reduced and good HDL cholesterol was preserved. There is

significant evidence that there is a link between monounsaturated fat and overall nut consumption and a lower risk of coronary heart disease. The majority of the fat in peanuts is heart-healthy monounsaturated fat with polyunsaturated and saturated fats in moderate amounts. Peanuts are the most abundant source of the protein “arginine” which contains all 20 amino acids in varying quantities. Peanut proteins and other legume proteins, such as soy proteins are nutritionally equal to meat and eggs for human growth and health, according to the Protein Digestibility Corrected Amino Acid Score (PDCAAS). Peanuts have a high digestibility of their components. When peanuts or peanut butter are added to a meal with a high glycemic load, such as a bagel and a glass of juice, blood sugar is regulated and does not climb too high too rapidly. Niacin is a vital nutrient for the digestive system, skin and nerves. It aids in the conversion of food to energy and is thought to protect against Alzheimer’s disease and cognitive decline. Copper, manganese, iron, phosphorus and magnesium are all found in groundnuts and their consumption is linked to reduced inflammation and a lower risk of metabolic syndrome. Numerous chemicals found in peanuts and their skins have been discovered to offer health benefits beyond simple nourishment. Peanuts are regarded as a functional meal with several functional components such as Coenzyme Q10, which protects the heart during periods of oxygen deprivation, such as high altitudes and clogged arteries. Peanuts are also high in dietary fibre and include a variety of critical nutrients, including many B vitamins, vitamin E, iron, zinc, potassium and magnesium, as well as antioxidant minerals (selenium, manganese and copper) and other antioxidant compounds (such as flavonoids and resveratrol). These bioactive components have been shown to have disease-fighting capabilities, with some acting as antioxidants and others promoting longevity. The total biological substances in peanut seed, such as vitamin E in oil or chlorogenic acid, caffeic acid, coumaric acid, ferulic acid, flavonoids and stilbenes, contribute to the antioxidant capacity (Arya *et al*., 2016).

Groundnut allergy is one of the most severe food allergies, affecting 1–2% of the world’s population. Australia is at the top of the list of countries that have been severely affected; other countries that have been severely affected include the United States, Canada, France, Denmark and the United Kingdom. Groundnut allergy is not only life-threatening but it also has a negative impact on the quality of life of groundnut-allergic people and their families. There is currently no vaccination to prevent groundnut allergy in susceptible persons, no medication to relieve allergic symptoms

and no techniques to minimize allergen proteins in groundnut products. There are 32 different types of storage proteins in groundnut seed and 18 of them are allergens (Ojiewo *et al*., 2020).

Uses of groundnut

The peanuts (seeds) are used for roasting, salting and making peanut butter. The filtered refined oil is used in cooking and in the production of margarine. Peanut oil is a vital food oil. The oilcake is fed to the animals as fodder. After hydrogenation, peanut oil is converted into vegetable ghee. In addition, the kernels are used in a variety of foods and confectionery. They're ground up and turned into peanut butter. Peanut flour is made by grinding the finest grades of peanut cake and is used to replace white flour. Cake is fed to cattle and other farm animals and it is also used as manure. Cake contains a lot of nutrients. Seed-coats are mixed with groundnut husk and the product is called groundnut bran.

The goodness of this legume is that it legumes can also be consumed in a completely different form -groundnut oil (also known as peanut oil). Peanut oil is used to make paint, varnish, lubricating oil, leather dressings, furniture polish, insecticides and nitroglycerin. Saponified oil is used to make soap, and peanut oil and its derivatives are found in many cosmetics. The protein component is used in the production of some textile fibres. Peanut shells are used in the production of plastic, wallboard, abrasives, fuel, cellulose (used in rayon and paper) and mucilage (glue).

Molecular markers

Groundnut genetic variability is low due to the crop's origins in a single hybridization event between two diploid species, followed by chromosome doubling and crossing barriers with wild diploid species (due to ploidy differences). The low genetic variability for important traits and the polyploidy nature of groundnuts are a bottleneck to groundnut improvement (Pasupuleti *et al*., 2013).

Because of the scarcity of polymorphic DNA markers in this crop, genomic research on cultivated peanut has lagged behind that of other crop species. Additional DNA markers must be identified for further genetic research in peanut. Microsatellites, also known as simple sequence repeats or SSRs, are a small array of tandemly arranged bases (one to six) that are distributed throughout the genomes. Microsatellites are more advantageous as DNA markers than many other markers because they are highly

polymorphic, abundant, codominant inheritance, analytically simple and easily transferable (He *et al*., 2003).

Molecular markers can be used to study genetic diversity and the relationships between plant species, populations, and individuals. The enormous appeal of RAPDs is that no DNA probes or sequence information are required for the design of specific primers. There are no blotting or hybridization steps in this procedure. As a result, the technique is quick, simple and efficient, requiring only the purchase of a thermocycling machine and an agarose gel apparatus to set up in a laboratory for any new system under investigation. It only requires a small amount of DNA (l0 ng per reaction) and the sample throughput can be quite high. The advantage of ISSRs is that no sequence data are required for primer construction. Groundnut has been identified as having a narrow genetic base and a low level of variation at the DNA level, as revealed by RAPD, ISSR, AFLP and SSR markers. The SSR marker has been the most successful of the major DNA marker types in identifying molecular variation within the cultivated groundnut species and good progress has been made in tagging economically-important traits in groundnut using RAPD, ISSR, SSR and SCAR markers.

Based on PCR Randomly Amplified Polymorphic DNA markers are useful genetic markers because they produce quick results, are inexpensive and need minimal oligonucleotide primers. With a minimal amount of template, a high number of fragments from various sections of the genome can be created, allowing several loci to be investigated fast (Ridhhi *et al*., 2016). Microsatellites, also known as simple sequence repeats or SSRs, are small array of tandemly arranged bases (one to six) found throughout the genome. Microsatellites are superior to many other DNA markers in that they are highly polymorphic, abundant, have co-dominant inheritance, are analytically simple and are easily transferable. Microsatellites have been shown to be more variable than RFLPs or RAPDs, and they have been widely used in plant genomic studies. When micro-satellites are employed to track desirable qualities in large-scale breeding programmes and as anchor points for map-based gene cloning procedures, the advantages of micro-satellites over other forms of genetic markers will become more essential and visible (He *et al*., 2003).

Germplasm collections aid in the identification of desirable parents for hybridization programmes. Because they are abundant, independent of tissue or

environmental effects, and allow cultivar identification early in plant development, molecular markers are a useful complement to morphological and physiological characterization of cultivars. Polymorphisms are detected more frequently by molecular markers based on differences in DNA sequences between individuals than by morphological and protein-based markers. To amplify regions between microsatellites, Simple Sequence Repeats (SSR) is used as a primer. This marker reveals far more fragments per primer than RAPD analysis (John *et al*., 2018).

ISSR are arbitrary multiloci markers generated by micro satellite primer and PCR amplification. They are useful since no genetic information about the primers is necessary to use them. These are semi-random indicators amplified by PCR using one primer that is complementary to the target micro satellite. ISSR markers, like RAPDs, are quick and simple to utilise and have been used in a variety of plant species. ISSR markers solve the shortcomings of RAPD's low repeatability, AFLP's high cost, SSR's complexity and they provide a quick and cost-effective approach (Sharma *et al*., 2017).

Practical utility of the research work:

The PCR-based marker techniques like RAPD, ISSR and SSR have been used for characterization of groundnut varieties. Molecular marker technologies are the effective tools and they are used for the assessment of genetic variability because they are not influenced by the environment. Molecular markers for the exploitation and identification of plant genetic diversity are one of the most key developments in the field of molecular genetics studies. The information may facilitate selection of genotypes to serve as parents for effective breeding program in crop improvement and also give information on varietal genetic diversity. This identified diverse genotypes can be used for creating new varieties. Different biochemical analysis of the groundnut varieties could be helpful in developing parental lines according to its biochemical characters in future breeding programs. The objectives to be envisaged are as under.

1. To analyse the groundnut varieties for the biochemical parameters.
2. To assess the groundnut varieties for genetic diversity using molecular markers.
3. To find out the phylogenic relationship among different groundnut varieties.

CHAPTER - II

REVIEW OF LITERATURE

An attempt has been made to bring together some of the recent reviews and findings on proximate parameters and molecular markers. The literature available on groundnut pertaining to objectives of this investigation entitle "Genetic diversity analysis in groundnut (*Arachis hypogaea* L.) based on molecular markers and biochemical parameters" has been reviewed as under.

2.1 BIOCHEMICAL PARAMETERS

2.2 MOLECULAR MARKERS

2.2.1 Randomized Amplified Polymorphic DNA (RAPD)

2.2.2 Inter Simple Sequence Repeats (ISSR)

2.2.3 Simple Sequence Repeats (SSR)

2.1 BIOCHEMICAL PARAMETERS

Eugene and Juliana (2003) studied the effects of heat processing methods on the proximate composition, caloric value, mineral concentrations, vitamins A and C levels and lipid characterization of seed pastes of *Arachis hypogaea*. Moisture content was the highest (25.2 ± 0.36%) in boiled seeds (BS) followed by raw seeds (RS) (4.58 ± 0.24%) and was the lowest (1.21 ± 0.20%) in fried seeds (FS), while ash ranged from 1.31 ± 0.01% in BS to 2.15 ± 0.07% in FS. Protein was highest in FS (28.45 + 0.21%) followed by RS (26.40 ± 0.18%) and lowest in BS (18.64 ± 0.22%). RS had the highest crude fat of 51.1 ± 0.16%, while BS had the lowest value of 25.23 ± 0.38%. Total carbohydrate was lowest in RS followed by FS and the highest value of 29.62 + 0.30% in BS. The energy content in kcal/100g sample was in the order RS>FS>BS. The seeds were found to be good sources of copper, iron, zinc, sulphate and chloride. Boiling and frying had no significant effect ($P>0.05$) on copper but significantly ($P<0.05$) decreased iron and zinc. Boiling significantly ($P<0.05$) decreased sulphate and chloride which were significantly increased by frying. Boiling decreased the concentrations of vitamins A and C which were further decreased by frying. The per cent free fatty acid, peroxide value, acid value and iodine value were highest in RS and the lowest values were obtained in FS, BS, FS and FS, respectively. No significant difference ($P>0.05$) was

obtained in the saponification number of the three samples analysed and values ranged from 161.3 ± 2.92 in RS to 163.0 ± 2.60 in FS. Heat processing (boiling and frying) generally decreased significantly ($p<0.05$) the crude protein, crude fat, caloric value, Fe, Zn, vitamins A and C as well as % free fatty acid and peroxide value but had no significant effect on Cu, acid value, iodine value and saponification number. However, frying increased significantly ($P<0.05$) the dry matter, ash, SO_4^{2-}, Cl^- and unsaponifiable matter. These findings may offer scientific basis for the use of the processed seeds as food for humans and oil extracts for the manufacture of industrial products.

Asibuo *et al*. (2008) determined nutritional quality of 20 groundnut varieties grown in Ghana. Dry samples were examined for oil content, crude protein, total carbohydrate, calcium, potassium, magnesium, sodium, zinc, copper, iron and manganese. The results of these studies showed that the parameters tested varied significantly. The oil content of virginia cultivars belonging to subspecies hypogaea was greater (49.7%) than that of spanish and valencia market kinds belonging to subspecies fastigiata (47.3 %). However, subspecies fastigiata had a greater mean protein content (25.69%) than subspecies hypogaea (22.78 %). A Spanish market type had the highest crude protein content (30.53%) and the least oil content (33.60%) and is idea for products which require more protein and less oil.

Atasie *et al*. (2009) did proximate, physico-chemical and elemental analysis of groundnut. The results showed that the groundnut oil contained 47.00% fat, 38.61% protein, 5.80% moisture, 1.81% carbohydrate, 3.70% crude fibre and 3.08% ash. Minerals (mg/100g) included: Na (42.00 ± 0.71), K (705.11 ± 0.86), Mg (3.98 ± 0.04), Ca (2.28 ± 1.94), Fe (6.97 ± 1.62), Zn (3.20 ± 0.11), P (10.55 ± 0.68). The physico-chemical characteristics showed; saponification value 193.20 (mgKOH/g), iodine value 38.71 (g/100g), acid value 5.99 (mgKOH/g), free fatty acid 3.01 (mgKOH/g), peroxide value 1.50 (meq/kg) and refractive index 1.449. The predominant fatty acid was found to be oleic acid (41.11%). The groundnut can thus be considered as a good source of protein with high nutritional value.

Campos *et al*. (2009) studied on six peanut cultivars for agricultural yield, chemical composition (protein, fat, carbohydrates, fiber and ash), amino acid profile, digestibility, fatty acid profile, tocopherol and sterol contents. Ranferi Daz and Col-61-

Gto had the maximum yield (6.3 Ton/ha), according to the results. Protein level ranged from 23.5% to 26.6%, with fat content ranging from 49.8% to 53.4%. The average digestibility was 86%. All cultivars have enough quantities of lysine and threonine to suit human needs. Saturated fatty acids in total varied from 15% to 18%. The oleic/linoleic ratio was calculated to be around 1.3-1.4. Tocopherol concentrations ranged from 390 to 706 ppm. The cultivars with the lowest yield had the greatest tocopherol levels. The alpha tocopherol level was assessed to be 90-150 parts per million, whereas the gamma tocopherol content was 270-570 ppm. The main sterol present was beta-sitosterol (approx. 65%). Ranferi Diaz variety presented the highest agronomic yield and the highest protein content but low oleic acid, low sterols and low total tocopherols. The differences among cultivars suggest differences in their applications.

Muhammad *et al.* (2009) determined biochemical composition and some phytochemicals in the seeds of 4 groundnut (*Arachis hypogaea* L.) varieties *viz*., Golden, Barri 2000, Mongphalla and Mongphalli 334 cultivated in arid zones of Pakistan. The biochemical analysis included ash, crude fat, total nitrogen, proteins and sugar contents. A statistically significant difference ($p<0.05$) was observed among the varieties regarding the ash, crude fat, water soluble proteins, salt soluble proteins and sugar contents. The four groundnut varieties were also found to be significantly different ($p<0.05$) on the basis of phytochemicals analysed including tannins (822 ± 3.78 to 903 ± 4.45 mg/100g), saponins (438 ± 2.12 to 480 ± 2.30 mg/100g), non-protein nitrogen (1.33 ± 0.03 to 1.56 ± 0.02 mg/100g), hydrogen cyanide (40.80 ± 0.32 to 42.82 ± 0.75 mg/100g), total phenolic acids (218 ± 2.11 to 256 ± 2.02 mg/100g), total phosphorus (700 ± 3.62 to 889 ± 3.84 mg/100g) and phytic acid (572 ± 4.37 to 714 ± 3.74 mg/100g). The results obtained from the present studies could be a source of valuable information and a guideline for the food scientists, researchers and even the nut consumers not only in Pakistan but all over the world.

Ingale and Shrivastav (2011) analysed proximate composition, anti-nutritional and nutritional value of seeds of new variety of groundnut (*Arachis hypogaea* L) JL-24 was determined. The groundnut seed included moisture (5.529%), crude fibre (1.149%), fat (46.224%), crude protein (25.20%), carbohydrate (21.26%), ash (2.577%), calcium (0.087%), phosphorus (0.29%) and energy (601.856%). For saturated and unsaturated fatty acids, the total fatty acid content was 10.44% and

33.51%, respectively. Protein solubility was measured at pH levels ranging from 0.5 to 13.5, with the largest amount of seeds proteins extracted at pH 12. The seed protein did not include serine and the seed was determined to have the largest level of proline (6.412%). The anti-nutritional study revealed cyanide concentration of 4.818 HCN/100 g, tannin of 0.412/100 g, oxalate of 0.180/100 g and haemagglutinin activity of 1:8 for goat blood group and no haemagglutinin activity for chicken and human blood groups, as well as no trypsin inhibition.

Wang *et al*. (2011) conducted a study to determine quality analysis of seed samples of 152 genotypes of groundnut. The study founded that the protein, oil and sucrose content, oleic acid and linoleic acid content, as % age of total fatty acids, ranged from 18.93 to 30.22%, 37.42 to 55.69%, 2.73 to 14.65%, 20 to 80.51% and 2.91 to 41.82%, respectively. The relationships between these quality attributes were also investigated. Protein content was unrelated to oil content, but both were negatively related to sucrose content. The study also suggested that developing high protein, oil or sucrose groundnut cultivars with a high oleate to linoleate ratio is feasible, as this ratio is unrelated to protein, oil or sucrose content. Germplasm lines with a high protein, oil, sucrose or oleate to linoleate ratio were identified, providing materials for studying quality trait genetics and breeding quality groundnut cultivars.

Ayoola *et al*. (2012) performed proximate and mineral analysis of groundnut (*Arachis hypogaea* L.) seeds on dry weight basis. The consequence showed that the groundnut seeds (raw, sun dried a roasted) contain moisture content of 7.40%, 3.40%, 1.07%; ash content of 1.48%, 1.38%, 1.41%; crude protein of 24.70%, 21.80%, 18.40%; crude fat of 46.10%, 43.80%, 40.60%; crude fibre of 2.83%, 2.43%, 2.41%; carbohydrate of 17.41%, 27.19%, 36.11%; respectively. Minerals included; sodium (0.71%, 0.69%, 0.57%), phosphorus (0.68%, 0.65%, 0.69%), potassium (0.47%, 0.51%, 0.55%), zinc (0.44%, 0.42%, 0.50%) and iron (0.40%, 0.47%, 0.43%). There was a general decrease in proximate composition after different heating methods, but there was variation in the mineral content of the seeds after heating. Based on the results, it is suggested that groundnut seeds be used in animal feeds (poultry), a complete human diet (balanced diet for elderly people who require little carbohydrate but a lot of protein) and as an antidote for malnourished children.

Noubissie *et al*. (2012) carried out a study in Dang, Cameroon's soudano-guinea zone, to investigate varietal differences in protein and oil content of kernels in 12 promising groundnut (*Arachis hypogaea* L.) genotypes, estimate heritability values through simple analysis of variance, and evaluate correlations with some physicochemical parameters. The field experiment used a randomised complete block design with six replicates. The oil and protein contents of the genotypes studied differed significantly ($p \leq 0.05$) according to the analysis of variance. The average protein content of kernels was found to be 19.28%, with an oil content of 52.83%, implying that for confectionary quality, high protein and low oil groundnut genotypes are required. These biochemical characteristics were moderately to highly heritable, with broad-sense heritability (h2) values of 0.52 (oil content) and 0.64 (protein content). Low expected genetic advance values, 3.70% for oil and 4.70% for protein, indicated a predominance of non-additive gene effects. Oil content was found to be positively correlated with kernel weight (r=0.67), surface area (r=0.63), degree of sphericity (r=0.77) and porosity (r=0.57), while protein content was found to be negatively correlated with oil content (r=-0.51) and physical properties investigated. According to the findings of this study, the protein and oil contents of kernel can be gradually improved through selection for confectionary quality.

Bhanu and Sadagopan (2013) analysed raw and roasted groundnut seeds for proximate and nutritionally valuable minerals to compare raw and roasted groundnut seeds. The results showed that the total ash content of raw groundnut seeds (4.6%) was higher than that of roasted groundnut seeds (4.1%). The highest (26.1%) content of crude protein recorded in roasted groundnut compared to that of raw groundnut (24.9%). Crude carbohydrates level of raw groundnut (25.3%) was lower when compared with that of roasted groundnut (26.5%). Crude fat ranged from 39.1% (raw groundnut) to 39.6% (roasted groundnut). Crude fiber percentag both in raw (2.9%) and roasted (3.1%) conditions were good. Because of the lack of heat exposure, the raw groundnut had a higher moisture content (4.1%) than the roasted groundnut (3.6%). In both raw and roasted conditions, seeds had higher energy values. Minerals such as potassium, calcium, magnesium, phosphorus and zinc were found in significant amounts in both raw and roasted conditions. Statistical analysis revealed highly significant differences ($P<0.05$) between raw and roasted seeds.

Yadav *et al*. (2014) evaluated nutritional quality of four varieties (RHRG-6021, RHRG-6083, TAG-24 and JL-501) of groundnut. The RHRG-6021 genotype had the highest oil content (51.65%). The oil content, iodine value, free fatty acid content, protein content and total polyphenol content of groundnut were all non-significant in the storage container. All groundnut genotypes had the highest iodine value after 270 days of storage. Significant differences in free fatty acid content were observed in all four groundnut genotypes, with the JL-501 genotype recording the highest free fatty acid content after 270 days of storage. At the start of storage, genotype TAG-24 had the highest protein content (24.85%). At 270 days of storage, the groundnut genotype RHRG-6083 had the highest polyphenol content (0.544), which increased subsequently during storage. The highest saponification value (198.2) recorded in JL-501 after 270 days of storage. The saponification value of the polylined HDPE bag was higher than that of the HDPE bag. The RHRG-6021 genotype has the highest initial total sugar (7.36%). The genotypes stored in polylined HDPE bags had a higher total sugar content than the genotypes stored in HDPE bags.

Chowdhury *et al*. (2015) conducted an experiment on five varieties growing in large scale in Bangladesh which were evolved by BARI. The maximum (128.3 g) seed weight was discovered in BARI Chinabadam-7, while the lowest (66.76 g) seed weight was discovered in Dhaka-1. The variety Dhaka-1 had the most moisture (5.120%), whereas BARI Chinabadam-9 had the least (1.230%). The variety BARI Chinabadam-8 had the largest quantity of ash (9.6%), whereas BARI Chinabadam-9 had the lowest amount of ash (7.8%). In this study, the BARI Chinabadam-6 had the greatest carbohydrate content (6.275%) while the BARI Chinabadam-8 had the lowest carbohydrate content (1.218%). The BARI Chinabadam-9 had the highest content of protein (38.88%) whereas, BARI Chinabadam-7 had the lowest protein content (36.60%). The variety Dhaka-1 contained the least quantity of oil (49.20%), but the variation BARI Chinabadam-9 contained much more oil (50.76%). The total energy content of these types ranged from 290.3 Kcl/gm to 317.7 Kcl/gm. Each variety contained the same quantity of saturated fatty acids (10.92% to 17.47%) and unsaturated fatty acids (81.13-94.81%). There was significant genetic variation for chemical composition and nutritional properties, which might be used for various food preparations and breeding purposes. It also depicts the usage of groundnut and

recommends a future plan for nutritionists, health counsellors, and dieticians on how to make the greatest use of groundnut.

Evans *et al*. (2015) analysed twenty-four (24) fresh and stored groundnut samples using standard methods and procedures. The consequence indicated average moisture, crude protein, crude fat, crude fibre, ash and carbohydrate contents of 3.65%, 24.78%, 44.70%, 5.65%, 2.18% and 19.16% for fresh groundnuts and 3.88%, 27.08%, 47.62%, 6.76%, 2.28% and 12.28% for stored groundnuts, respectively. After storage, 83.33%, 58.33% and 66.67% of the samples recorded increase in moisture and crude protein, fibre and ash content, and crude fat correspondingly. However, carbohydrate levels in 91.67% of the samples had decreased following storage. Fertilization had no significant influence on the proximate composition of either category of groundnuts; hence, using these fertilisers to increase groundnut proximate composition may not be recommended; nevertheless, storage may improve proximate composition.

Kulkarni *et al*. (2015) evaluated sixteen low temperature stress tolerant groundnut genotypes for the levels of crude fat, crude protein, reducing sugar, non-reducing sugar and total sugars from the defatted meals. Out of 16 genotypes, four genotypes *viz*., NRCG 1613, NRCG 9799, NRCG 12212 and NRCG 2510 germinated at 15°C, and the levels of proline and extracted soluble proteins from leaf material were resolved on a denaturing polyacrylamide gel to see if there were any variations in banding patterns under stressful conditions. The seeds of the low temperature stress resistant genotype ICG 6888 had the greatest fat content (52.46%), followed by NRCG 9799 at 49%. Among the 16 genotypes, ICG 1298 and ICG 6570 had the greatest crude protein content (38.5%) in their defatted meals, followed by ICG 7005. The reducing sugar concentration in defatted groundnut seeds was also measured, with genotype ICG 1256 having the greatest reducing sugar content of 1.60%, followed by ICG 3942 with 1.40%. ICG 1298 had the highest total sugar content of 9%, followed by ICG 1256 at 8.10%.

Mora-Escobedo *et al*. (2015) made study on eight peanut (*Arachis hypogaea*) cultivars that were grown in Mexico and were analyzed for the physical and chemical characteristics of their seeds and for the physicochemical properties and fatty acid profiles of their oils to select the most promising candidate in terms of oil stability and nutrient composition. The results showed that the protein ranged from 28.5% to 32.9%

and the oil varied from 37.9% to 56.3%. The major fatty acids found in the oil samples were palmitic (11.9-13.2%), oleic (45.2-53.8%) and linoleic (25.1-29.2%) acids. The oleic/linoleic ratio was between 1.8 and 2.1. The physicochemical characteristics under evaluation were as the iodine value (88.6-105.4), saponification value (142.5-181.8) and acidity (1.1-2.5%).

Sujatha *et al*. (2018) arranged an experiment to evaluate the biochemical changes in the groundnut seeds stored in different locations, storage conditions and containers during 12 months of storage. According to the study results, Bagalkot produced seeds stored in cold storage conditions and packed in vacuum pack maintained higher seed quality parameters such as higher oil content (47.78%), protein content (31.19%), and lower lipase (0.53 moles min^{-1}) and protease activity (0.262 U/ml) after 12 months of storage as compared to Dharwad and Kumta locations, ambient condition and other containers. With the passage of time, oil and protein levels dropped but lipase and protease activity rose. It was found that cold storage conditions and vacuum packing should be employed for groundnut seed storage in order to retain seed viability for a longer length of time.

Shashikant (2019) studied four different groundnut varieties such as UF70-130 (Uf70-130), Kopargaon-1 (K-1), Kopargaon-3 (K-3) of Maharashtra and RSB-103-87 (Rsb-103-87) of Rajasthan grown under different geographical places in India. For the nutritional assessment, the proximate analysis, oil production and physicochemical parameters of harvested groundnut seeds and their extracted oils were assessed. It was found that the seeds contained extracted oil, moisture, crude protein, total ash, crude fat, crude fibre, carbohydrate and calories per 100gms in the range of 39.45-41.48% (± 0.8603), 1.68-2.48% (± 0.3311), 28.75-30.63% (± 0.8082), 1.39-2.02% (± 0.262), 28.68-30.15% (± 0.653), 1.69-2.32% (± 0.267), 33.77-36.29% (± 1.029) and 520.8-526.43 (± 2.633), respectively. The specific gravity, viscosities, impurities and refractive index of the extracted groundnut seed oil and colour were in the range of 0.9149-0.9162 (± 0.0006), 91.5-91.6 (± 0.0447), 0.035-0.06 (± 0.0104), 1.4628-1.4636 (± 0.0003) and 1.12-2.1 (± 0.425), respectively. AV (mgKOH/g oil), IV (gI2/100 g oil), SV (mgKOH/ g oil), unsaponifiable matter content (%) and ester value of the extracted oil from groundnut seeds were in the range of 3.17-4.29 (± 0.5117), 89.14-93.9 (± 2.2065), 190.08-192.47 (± 0.9783), 0.54-0.65 (± 0.052) and 185.87-188.5 (± 1.1574),

respectively. This was an empirical study, and the results demonstrated that groundnut seed oil may be a good source of edible oil.

Oluwasegun *et al*. (2020) determined proximate and some essential mineral compositions of groundnuts processed by different methods for consumption. The parameters were analysed using standard methods of the Association of Official Analytical Chemists (AOAC, 2005) and Greenfield and Southgate, 2003. From the results obtained, it was found that the proximate compositions (%) of the groundnuts boiled with the pods were contained moisture content (18.77 ± 0.02), crude protein (22.93 ± 0.06), crude fat (38.94 ± 0.03), crude fiber (1.75 ± 0.02), ash (3.90 ± 0.04) and carbohydrate (13.73 ± 0.04). Groundnuts roasted with the pods had moisture content (2.91 ± 0.04), crude protein (29.73 ± 0.08), crude fat (50.11 ± 0.04), crude fiber (4.36 ± 0.03), ash (5.14 ± 0.03) and carbohydrate (7.76 ± 0.05), while those of the groundnuts roasted without pods were as moisture content (2.88 ± 0.04), crude protein (26.64 ± 0.06), crude fat (49.04 ± 0.02), crude fiber (4.26 ± 0.03), ash (4.73 ± 0.02) and the carbohydrate (11.50 ± 0.04). Na (0.23), K (0.67), Ca (0.11), P (0.38), Mg (0.16) and Fe (41.5) were the mineral compositions (in mg/kg) of the groundnut sample cooked with pods. Na (0.38), K (0.98), Ca (0.17), P (0.47), Mg (0.27) and Fe (61.2) were found in groundnut samples roasted with pods, while Na (0.27), K (0.82), Ca (0.16), P (0.42), Mg (0.25) and Fe (61.2) were found in groundnut samples roasted without pods (53.6). According to this study, the proximate and the mineral contents in the roasted samples were generally higher than those of the boiled sample except for the moisture and the carbohydrate contents.

2.2 MOLECULAR MARKERS

Related reviews on the work done on molecular characterization of groundnut have been presented under.

2.2.1 Randomized Amplified Polymorphic DNA (RAPD)

Subramanian *et al*. (2000) worked on 48 RAPD markers to characterize the genetic diversity of seventy groundnut genotypes. Only 7 (14.6%) of the 48 oligonucleotide primers produced polymorphic amplification products. The total number of bands produced by the seven primers was 408, including 27 of them being polymorphic. The discovery of polymorphism in farmed groundnut provides the door

to the construction of its genetic map through the careful selection of genotypes with DNA polymorphism.

Dwivedi *et al*. (2001) did assessment of genetic diversity using germplasm with different DNA profiles. The current work aimed to explore molecular diversity among chosen groundnut accessions and to identify those with different DNA profiles for mapping and genetic improvement. For the random amplified polymorphic DNA experiment, 26 accessions and eight decamer primers were used. The genetic similarity (Sij) varied from 59% to 98.8%, with an average of 86.2%. Dendrograms using multidimensional scaling and the unweighted pair-group method with arithmetic averages (UPGMA) revealed the presence of five distinct clusters. This categorization, however, could not be linked to known biological information about the accessions classified into separate clusters. Some groundnut accessions with different DNA profiles (ICG 1448, 7101 and 1471, as well as ICGV 99006 and 99014) were discovered for mapping and genetic improvement.

Lang and Hang (2007) demonstrated the utility of random amplified polymorphic DNAs (RAPDs) to analyse genetic divergence of peanut genotypes in the South Vietnam. Nucleic acid extracts from 29 *Arachis hypogea* L. cultivars were amplified with five random decamers by PCR. RAPD 2, RAPD 3, RAPD 5, RAPD 6 and OPC 11 were utilised as markers. The different RAPD patterns created by these cultivars might be utilised as a genetic fingerprint to determine a genotype's identification. In a phyllogram derived by unweighted pair group method analysis (UPGMA) of genetic distances, 29 peanuts were clearly split into discrete subclusters. The phyllogram, which was developed using UPGM analysis of the genetic distances, depicts the numerous connections among these cultivars. OMDP 17, OMDP 7 and OMDP 4 were all connected in some way. This is also indicated by the great similarity in the flower structure and color patterns of these three cultivars.

Vyas *et al*. (2014) investigated genetic diversity among fifteen genotypes of groundnut by random amplified polymorphic DNA (RAPD) analysis. A total of 15 primers were employed to identify polymorphism, however only 13 of them indicated amplification. Out of the 13 amplified primers, 11 showed varying degrees of polymorphism ranging from 25% (S-31) to 100% (OPD-02), while two primers, OPA-02 and S-67, showed monomorphism. Thirteen RAPD primers amplified 54 fragments, with 28 of them being polymorphic (51.85%). The number of fragments amplified per

primer ranged from 3 to 5, with sizes ranging from ~150 bp to ~1800 bp. The cluster analysis classified fifteen groundnut genotypes into six major groupings. The pairwise similarity values ranged from 76% to 94% showed that genotypes UG-109 and UG-110 were the closest with highest similarity value (94%), while genotypes UG-100 and GG-7 were most distinct with minimum similarity value (69%).

Al-Saghir and Abdel-Salam (2015) evaluated the genetic diversity of the peanut accessions using Random Amplified Polymorphic DNA (RAPD) molecular marker. This study included twenty peanut accessions that were analysed using RAPD molecular markers. Twenty-seven RAPD primers resulted in 210 amplification products, with 80 (36.4%) of them being polymorphic. In conclusion, their work established the use of RAPD markers in determining the level of genetic diversity in peanut germplasm by successfully fingerprinting peanut accessions using these markers.

El-Akkad (2015) evaluated RAPD, AFLP and SDS protein marker systems for their ability to detect genetic diversity among ten peanut (*Arachis hypogaea* L.) genotypes and to compare the efficiency of these marker types in the classification of accessions according to the gene pool of peanut genotypes. The polymorphic fragments were obtained using a combination of 10 RAPD differentiating primers, 2 AFLP differentiating primer combinations and SDS protein. The ten RAPD primers yielded 38 polymorphic bands, whereas the AFLP primer combinations yielded 30 polymorphic bands and the SDS protein pattern yielded four. The genetic similarity between ten peanut accessions ranged from 75.3% to 98.7% according to RAPD data analysis, while the highest genetic similarity value was 97.8% and the lowest value was 74.7% according to AFLP data, and the highest genetic similarity value was 100% and the lowest value was 71.4% according to SDS protein data. The dendrogram generated with hierarchical UPGMA (Un-weighted Pair Group Method with Arithmetic Averages) cluster analysis of the Jaccard's similarity coefficient matrices revealed two major clusters, which were identified.

Patel *et al*. (2015) used RAPD showing reproducible and polymorphic patterns were chosen for groundnut cultivar identification and generated a total of 103 bands/alleles in which 96 bands were polymorphic (not present in at least one variety out of 12 varieties used) that was equal to 93.32% polymorphism and 7 bands were monomorphic (present in all 12 groundnut varieties). The average bands per primer

were 4.68. The PIC values varied between 0.279 (OPB-09) and 0.878 (OPC-13) with an average of 0.661 per primer. Among the screened primers OPA-05, OPA-10 OPA-15 and OPB 11 showed variety specific markers, while OPB-09, OPB-11 OPC 11, OPL 12 and OPO-11 produced species specific DNA fragments. The dendrogram generated by the UPGMA method produced two main clusters, each of which contained all of the varieties grouped together in their respective sub-cluster.

Suneetha *et al*. (2015) examined genetic diversity among 12 genotypes of groundnut using RAPD markers. Seven polymorphic RAPD markers were discovered among the 60 RAPD markers analyzed. In all, 58 amplification products were discovered, with 48 alleles being polymorphic (82.9%). OPJ 6 was the most polymorphic among all the markers, with 11 alleles, nine of which were polymorphic. The OPJ 4 marker amplified the fewest number of alleles. The examination of Jaccards similarity co-efficients among genotypes indicated that TCGS 645 and Tirupati 4 were distant, but Narayani and Kalahasti were quite similar (92.9%). To discover genotype-specific alleles, OPA3 for Tirupati 4 and TCGS 888, OPA 19 for Tirupati 3, TG 47 and TCGS 913, OPJ4 for Tirupati 3 and Prasuna, and OPJ 6 for Kalahasti and TCGS 750 were employed. The study demonstrated the scope and utility of RAPD markers in groundnut diversity analysis and genotype identification.

Kushwah *et al*. (2016) worked on Random Amplified Polymorphic DNA (RAPD) markers to create molecular genetic fingerprints of 14 groundnut germplasms in order to determine genetic diversity/relatedness among the species. Among the 14 genotypes, a total of 27 bands ranging in size from 200 bp to 2250 bp were detected, and all 27 (100%) were found to be polymorphic. Jaccard's pairwise similarity coefficients were used to determine the genetic relationship between groundnut genotypes. The pairwise similarity ranged from 0.00 to 0.833. The highest similarity coefficient was found between 'Dh-204' and 'Dh-209' (0.833), suggesting that they have a similar genetic background, whereas the lowest similarity was found between 'ICGV-95401' and 'DhS-102' (0.067). A dendrogram was plotted for the data set after data analysis using Jaccard's similarity coefficient. It showed that at genetic distance 0.00, two separate clusters with a single root were produced. The dendrogram revealed two large clusters (I-II) that were determined to be separate from each other, with just 0% similarity. Cluster I had the most genotypes (13), whereas Cluster II had only one. The results of principle component analysis were very similar to the results of UPGMA.

Scaling in two and three dimensions revealed four clusters (I to IV). Cluster-I had six accession, Cluster-II had three, Cluster-III had four and Cluster-IV had only one accession. According to the findings, the wide range of similarity values for related accessions utilizing RAPD gives better confidence in assessing genetic diversity and connections.

Kusuma *et al*. (2016) estimated the genetic diversity and evaluated the relationship among eight groundnut genotypes using Random Amplified Polymorphic DNA (RAPD). This study included eight groundnut genotypes that were analyzed using RAPD. A total of ten primers were employed to identify polymorphism, however only eight of them exhibited amplification. The primers used yielded 52 amplification products, 34 of which were polymorphic (65.38%). The number of fragments amplified by each primer ranged from 2 to 15, with sizes ranging from ~500bp to ~2000bp. The OPD-02 primer produced the greatest number of amplification products (100% polymorphism). UPGMA cluster analysis found that the genotypes Narayani and Dharani were genetically related (86.8% similar), whereas Kadiri-6 and Dharani were dissimilar. Amplification patterns obtained with certain primers might aid in the identification of genotypes resistant to biotic, abiotic and agronomically crucial factors. The alleles represented in 8 groundnut genotypes with 5 RAPD markers must be validated across a wide variety of genotypes.

Shamha *et al*. (2019) evaluated the genetic diversity of five peanut cultivars grown under field conditions. During two subsequent seasons, a field experiment was conducted using five peanut cultivars (Giza-5, Giza-6, Ismailia-1, Gregory and R92) in a randomised complete block design with five replications to estimate the performance of five peanut cultivars for vegetative growth, yield, yield component traits and seed quality traits. For each of the five cultivars, twenty RAPD primers were utilised to identify a unique fingerprint. The Giza-6 cultivar outperformed all other peanut cultivars in terms of vegetative growth features, yield and component attributes, whereas the Giza-5 cultivar had the lowest results. The dendrogram constructed from RAPD analysis showed that Gregory and Giza-5 were the most distant among five peanut cultivars.

Suryadi *et al*. (2019) assessed genetic diversity of peanut cultivars, i.e., Jerapah, Kancil and Hypoma 2, based on RAPD markers. Three individuals of each cultivar were tested using the PCR-RAPD approach, which included twelve primers: OPA-1,

OPA-2, OPA-9, OPA-13, OPB-2, OPB-3, OPB-4, OPB-5, OPB-7, OPB-11, OPB-12 and OPJ-7. There also included data analysis based on morphological data. Only 7.55% of the bands were polymorphic, whereas the majority of the bands were monomorphic, showing relatively little diversity across the cultivars. Kancil was closer to Jerapah cultivar in the phenogram based on literature, but Hypoma 2 was closer to Kancil cultivar in the RAPD-based dendrogram.

Vivekanand *et al*. (2019) used twenty four genotypes of *Arachis hypogaea* (L.), of which 12 genotypes belonging to Virginia and 12 belonging to Spanish varieties to study the genetic divergence within its botanical varieties using RAPD. The study involved 16 primers from the OPH family. In the Virginia and Spanish groups, 36 and 37 bands were obtained from the 16 primers used, respectively. For the Virginia and Spanish groups, respectively, 18 and 20 bands were polymorphic out of the total bands generated. The similarity coefficient for the Virginia group varied from 0.09 to 0.78, whereas it ranged from 0.13 to 0.88 for the Spanish group. For 24 groundnut genotypes, a dendrogram was created using the similarity matrix value generated from RAPD data. The dendrogram clearly separated 12 Virginia groundnut genotypes into four and five groups, respectively. In the future groundnut enhancement programme, the genetic linkages assessed might be valuable for hybridization.

2.2.2 Inter Simple Sequence Repeats (ISSR)

Mondal *et al*. (2009) investigated genetic diversity among twenty cultivated groundnut (*Arachis hypogaea* L.) genotypes using inter simple sequence repeat (ISSR) markers. Twenty-one ISSR primers were found to be polymorphic (74.67 %). The 3'-anchored primers based on the poly 'GA' and poly 'AG' motifs yielded a larger amount of polymorphism (74.85 and 77.27%, respectively). Because it had the best resolving capacity among the 21 primers, the primer UBC 810 recognized 18 genotypes out of the 20 examined. The Ward method of cluster analysis yielded four subclusters, with subcluster A and subcluster D containing resistant and susceptible genotypes, respectively. The UBC 810540 was linked with both rust and late leaf spot (LLS) resistance, while UBC 810500 was associated with LLS resistance, according to Kruskal- Wallis one-way ANOVA.

Tshilenge *et al*. (2012) assessed inter-simple sequence repeat (ISSR) markers to examine genetic heterogeneity among groundnut accessions from a gene pool. In

selected accessions, the influence of gamma ray radiation on molecular variation was also studied. Polymorphic loci, Nei's gene diversity (h), and Shannon's index (I) were all at 54%, 0.18 and 0.28, respectively, among accessions. Groundnut accessions were genetically related, with genetic distance values ranging from 0.11 to 0.37. When 0.10 KGy gamma-ray treatments were used on the JL 24 accession from Brazil, the amplitude of variation based on the level of polymorphic loci increased by 37% compared to the control. For the same therapy, gene diversity and the Shannon index grew by 84 and 57%, respectively (0.1 KGy).

Patel *et al*. (2015) studied nine ISSR primers, out of the ten generated 71 bands/alleles of which 58 bands were polymorphic that was equal to 79.70% polymorphism and 13 bands were monomorphic with an average of 7.88 bands per primer. The highest PIC value 0.9 was noticed with HB-18, while lowest PIC value 0.7 was recorded with I-28 with an average of 0.833 per primer. The varietal and species-specific markers were also observed with the ISSR primer HB-15.

Sharma *et al*. (2017) worked on DNA-based markers provide a reliable means of estimating the genetic relationships between genotypes and taxonomic groups as compared to morphological markers. For PCR amplification, twenty ISSR primers were used, with 18 primers amplifying. The Inter Simple Sequence Repeat (ISSR) research revealed a significant amount of polymorphism (85.71). Using 18 ISSR primers, a total of 77 amplified bands were produced, with 67 of them being polymorphic. PIC values varied from 0.07 to 0.37, with an average of 0.300 seen across all groundnut genotypes. For ISSR primers, Jaccard's similarity coefficient values varied from 0.54-0.90, with an average of 0.72. The majority of genotypes could be split into five primary groups using the dendrogram created by the UPGMA algorithm and PCA. Clusters I, II and IV each had two genotypes, whereas Cluster III had nine genotypes. Cluster V had five genotypes. However, genotypes UG162 and UG158, UG162 and UG182, and PM2 and UG 158 were determined to be genetically varied, with a minimum similarity value of 0.59, followed by genotypes UG162 and UG182, PM2 and UG 158, which had a similarity value of 0.61.

Mohammed *et al*. (2022) worked on genetic variability and diversity of 43 accessions of *A. hypogaea* collected from different regions of Ethiopia using ISSR markers. Out of 56 reproducible bands generated, 29 (51.8%) were polymorphic. The band size ranged from 120 bp to 1100 bp. The polymorphic information content (PIC)

value ranged from 0.29 to 0.76 with an average value 0.49. The mean Nei's gene diversity and Shannon's information index were 0.25 and 0.33, respectively. Genetic relationship between *A. hypogaea* accessions based on Jaccard's pair wise similarity coefficients varied from 44 to 83% with an average value of 63.5%. The UPGMA analysis grouped *A. hypogaea* accessions into five distinct clusters at 63.5% similarity coefficient, and the principal coordinate analysis revealed similar grouping.

2.2.3 Simple Sequence Repeats (SSR)

Hopkins *et al.* (1999) investigated simple sequence repeat (SSR) markers in cultivated peanut and to test these markers for their ability to discriminate among accessions. Peanut total genomic DNA libraries were created and screened using ^{32}P-labeled dinucleotide repeats $(GT)_{10}$ and $(CT)_{10}$. DNA sequences were obtained from SSR-containing clones and primer pairs were designed based on DNA sequences flanking the repeat motif when possible. Primer pairs were tested in polymerase chain reaction (PCR) assays with a collection of 22 peanut DNAs representing both cultivated and wild species. Six SSR markers, five from the library screening procedure and one from a search of publicly available DNA sequences, detected polymorphisms in the peanut DNAs. The discrimination power of cultivated peanuts was high, with 17 distinct genotypes represented among the 19 accessions tested. Each SSR marker amplified 2 to 14 DNA fragments, and the six markers as a group may amplify up to ten putative SSR loci. The SSR markers identified in this study were more effective than all other DNA-based markers tested to date in detecting molecular variation in cultivated peanut.

Krishna *et al.* (2004) identified and understood molecular genetic diversity in farmed peanut kinds, which can aid in successful genetic conservation as well as efficient breeding programmes in this crop. The New Mexico breeding program has embarked upon a program of improvement of Valencia peanut (belonging to the sub species fastigiata), because efforts to improve the yield potential are lacking due to lack of identified divergent exotic types. This study demonstrated molecular diversity using microsatellite markers in farmed Valencia peanut (sub spp. fastigiata) from throughout the world for the first time. In this work, 48 cultivated Valencia peanut genotypes were chosen and investigated using 18 fluorescently labelled SSR (f-SSR) primer pairs. Among the genotypes examined, these primer pairs amplified 120 polymorphic loci and amplified from 3 to 19 alleles, with an average of 6.9 alleles per primer pair. Cluster

techniques and principal component analysis were used to further examine the f-SSR marker data. The results revealed that (1) significant genetic variations were discovered among the analysed genotypes; (2) f-SSR-based clustering could identify the putative pedigree types of the current Valencia types of diverse origins; and (3) f-SSR in general is sufficient to obtain estimates of genetic divergence for the material under study.

Moretzoshn *et al*. (2004) developed 67 new microsatellite markers for *Arachis*. However, only three of these markers were polymorphic in cultivated peanut. These three new markers, along with five previously identified markers, were used to assess the number of alleles per locus and gene diversity in 60 *A. hypogaea* accessions. Using allelic variation observed in a selected set of 12 SSR markers, genetic relationships between these 60 accessions and a sample of 36 wild accessions representative of section *Arachis* were estimated. SSR markers revealed that the Brazilian peanut germplasm collection has high levels of genetic diversity. *A. hypogaea* accessions were assigned to similarity groups, which is a useful criterion for selecting parental plants for crop improvement. Microsatellite marker transferability was up to 76% for *Arachis* species, but only 45% for the other eight *Arachis* sections tested. A new marker (Ah-041) demonstrated 100% transferability and could be used to differentiate between AA and non-AA genome carriers in peanut accessions. The level of polymorphism observed among *A. hypogaea* accessions evaluated with newly created microsatellite markers was modest, correlating with the accumulated data that suggest that farmed peanut has a relatively low level of diversity at the DNA level. A panel of SSR markers was used to divide *A. hypogaea* accessions into two primary groupings. Identification of similarity groups could aid in the selection of parental plants for use in breeding operations. The transferability of markers between *Arachis* accessions was relatively high. Because the production of microsatellite markers is still expensive and time intensive, the ability to use microsatellite markers developed for one species in genetic evaluation of other species considerably reduces the cost of the research. The SSR markers created in this study could be particularly valuable for genetic studies of wild *Arachis* species, such as comparative genome mapping, population genetic structure, and species phylogenetic inferences.

He *et al*. (2005) developed 130 peanut simple sequence repeat (SSR) markers, 38 of which were chosen in this investigation because of their capacity to identify genetic variability among 24 peanut accessions. Eight SSR markers were discovered to

be effective in classifying plant types. Six SSR markers were unique to the botanical variations *fastigiata* and *vulgaris*, one to the botanical varieties *hypogaea* and *hirsuta*, and one to the botanical varieties *peruviana* and *aequatoriana*. Three of these, which were obtained from peanut expressed sequence tags (ESTs), were also linked to potential genes. As botanical varieties range in morphological attributes and correspond to various subspecies of *A. hypogaea*, these markers might be linked to genes involved in morphological trait expression. The findings also revealed that SSRs (also known as microsatellites) may play a role in determining cultivated peanut evolution. Multiplex PCR of botanical variety-specific markers might be used to aid in the genotyping of peanut lines.

Hoshino *et al*. (2006) reported on the testing of fifteen microsatellite primer pairs in 76 accessions of 34 species from the nine *Arachis* sections. The data showed that heterologous primers were very useful in *Arachis* because they had high transferability among species (91.0%) and allowed the amplification of very polymorphic putative loci, allowing both the characterization of most accessions and demonstrating high variability, even when represented by few accessions.

Tang *et al*. (2007) worked on to evaluate the genetic diversity of twenty-four accessions of peanut using thirty-four SSR markers. Ten to sixteen pairs of SSR primers indicated polymorphisms among the examined accessions. In the studied accessions, the greatest differentiation index, which was defined as the degree of genetic differentiation, reached 0.992. A unique set of polymorphic SSR primers was used to differentiate each accession, and the intra-variety genetic distance was calculated across accessions, with an average of 0.59 in var. *fastigiata*, 0.46 for the var. *hypogaea*, 0.38 for the var. *vulgaris*, and 0.17 for the var. *hirsuita*. For the four botanical types, a dendrogram based on genetic distances was built, revealing the existence of various clusters. The study indicated that there was a lot of intra-variety SSR polymorphism, and when more SSR markers are generated, the inherent genetic diversity will be discovered, and the building of a genetic map and marker-assisted selection for farmed peanut will be possible.

Cuc *et al*. (2008) constructed microsatellite-enriched library from the genotype TMV2. A total of 490 SSRs were discovered after sequencing 720 putative SSR-positive clones from a total of 3072. The 71.2% of these SSRs were of the perfect type,

13.1% were of the imperfect type, and 15.7% were compound. The GT/CA repeat motifs were the most common (37.6%) among these SSRs, followed by the GA/CT repeat motifs (25.9%). The primer pairs were initially optimised on two genotypes and could be designed for a total of 170 SSRs. Among 32 cultivated groundnut genotypes, 104 (61.2%) primer pairs produced a scoreable amplicon, and 46 (44.2%) primers revealed polymorphism. Polymorphic SSR markers detected 2 to 5 alleles per locus, with an average of 2.44 detected per locus. For these markers, the polymorphic information content (PIC) ranged from 0.12 to 0.75, with an average of 0.46. A phenogram was created to understand the relationships between the 32 genotypes based on 112 alleles obtained from 46 markers. The majority of genotypes representing subspecies hypogaea were grouped together in one cluster, whereas genotypes representing subspecies fastigiata were grouped primarily in two clusters. A new set of 104 SSR markers has been developed to expand the repertoire of SSR markers for cultivated groundnut. These markers had a high PIC value in cultivated germplasm, making them ideal for germplasm analysis, linkage mapping, diversity studies and phylogenetic relationships in cultivated groundnuts and related *Arachis* species.

Islam *et al*. (2008) utilized groundnut microsatellite markers in 23 elite groundnut genotypes to assess the genetic diversity. A total of 13 alleles were detected at 3 loci using the PM3, PM50 and PM238 microsatellite primer pairs. The number of alleles per locus ranged from 4 to 5, with an average of 4.33. Allele sizes in 23 genotypes varied from 137 to 217 bp, with SSR allele frequencies ranging from 0.022 to 0.500. The genetic distance between all 253 genotype combinations of groundnuts varied from 0.000 to 2.093, with an average of 0.92. The polymorphic information content (PIC) values varied from 0.617 to 0.701 and the primer PM3 was shown to be the most polymorphic. Based on Nei's (1972) genetic distance, the UPGMA dendrogram divided the aforementioned groundnut genotypes into two primary groups (I and II). Cluster I had two sub-clusters la and lb and cluster II consisted of two genotypes namely, ICGV 94165 and ICGV 00340 were unique and diversed from all other genotypes belonging to cluster I. Regarding 3 primer pairs, 4 specific alleles (PM3/195, PM50/146, PM50/137 and PM238/171) were able to distinguish a maximum of 6 genotypes and finally 2 (ICGV 94165 and ICGV 00340) from the above 23 groundnut genotypes. This method will be beneficial for identifying polymorphism,

which will lead to genotype identification and conservation of commercially developed groundnut varieties, as well as measuring genetic diversity, using SSR markers.

Naito *et al*. (2008) assessed the diversity and genetic relationships within the peanut germplasms in Japan using allelic variation in a selected set of 13 SSR markers. They examined 201 *A. hypogaea* accessions and 13 *Arachis* wild species accessions: 13 primer pairs amplified 108 polymorphic alleles in *A. hypogaea*. The discovered alleles ranged from 3 to 15, with an average of 8.3 per marker. The phenogram was created using the SSR genotypes. *A. hypogaea* and *A. monticola* formed a distinct group from diploid species, and they were divided into 150 genotypes. *A. hypogaea* and *A. monticola* were further split into two groups: the first group comprised primarily of spp. *fastigiata* accessions, while the second group primarily of spp. *hypogaea* accessions and tetraploid wild peanut *A. monticola*.

Gautami *et al*. (2009) investigated the germplasm analysis and interspecific transferability of a novel collection of groundnut SSR markers. A total of 29 SSR primer pairs were created, with 14 (61%) of them yielding scoreable amplicons. Only 8 (57%) of the 14 SSR primer pairs found polymorphism among the 23 lines. Six markers were used to record the monomorphic bands. A total of 18 alleles were amplified by the polymorphic markers (8), with an average of 2.3 alleles per locus. With an average of 0.25, the greatest PIC value (0.36) was found in primer pair ICGM01A11c, while the lowest (0.13) was found in ICGM01A04bto. Peanut germplasm was separated into four clusters by the dendrogram. Clusters I and II were made up entirely of cultivated tetraploid accessions, Cluster III was made up entirely of amphidiploids, and Cluster IV was made up entirely of wild (diploid) accessions. Except for IAC-R886, which was a Brazilian accession, Cluster I consist of eight cultivated tetraploid accessions from India.

Li *et al*. (2011) selected 709 SSR markers from public databases, of which 556 passed an initial test and were used to describe 16 peanut (*Arachis hypogaea*) genotypes. PIC (polymorphism information content) scores and heterozygosity indices were calculated for each marker to assess the genetic diversity revealed by SSR markers, and genetic distances were estimated from shared allele distances for the construction of a cladogram using the Neighbor-Joining method to illustrate the genetic relationships among the genotypes. In these genotypes, 235 (42.27%) of the markers

had polymorphisms. The average heterozygosity assessed from these 556 SSRs was 0.225, ranging from 0 to 0.992, and the average PIC was 0.209. With a range of 1 to 13, the average number of alleles per SSR was 2.5. However, 410 SSR sites possessed only one allele, demonstrating that cultivated peanut diversity was quite low. Dinucleotide GA repeat motif markers accounted for 26.4% of the polymorphic SSR markers, followed by dinucleotide CT (10.4%) and trinucleotide TAA markers (9.6%). The most common forms of SSRs were dinucleotide and trinucleotide repeat motifs, and the dinucleotide GA repeat motif had a greater variability than other varieties. The genetic links indicated by the cladogram were consistent with the pedigrees and origins of the studied peanut genotypes, demonstrating that these SSR markers may be used to assess genetic diversity in peanuts.

Yi *et al*. (2011) made study on identified peanut into three plant types, virginia (var. *hypogaea*), spanish (var. *vulgaris*), and valencia (var. *fastigiata*). The former is associated with ssp. *hypogaea*, whereas the latter two are associated with ssp. *fastigiata*. Twenty SSR markers were employed to examine the genetic diversity of three different groups of *hypogaea*, *vulgaris* and *fastigiata*. Ten pairs of SSR primers exhibited polymorphisms among the variety-specific SSR primers used in this study. A unique set of polymorphic SSR primers was used to identify each accession, and allele number was calculated among accessions, with an average of 6.7 in var. *hypogaea* and 5.4 in var. *vulgaris* and *fastigiata*. Gene diversity varied from 0.336 to 0.844 and PIC (polymorphism information contents) ranged from 0.324 to 0.827 for evaluation of genetic diversity. Dendrograms based on genetic distances were created, revealing the existence of three distinct groups, and these three distinct clusters might be linked to the genes involved in three different plant kinds. The findings also revealed that SSR polymorphisms were abundant among peanut germplasm accessions in the RDA (Rural Development Administration, Korea) Genebank and that SSRs might play an essential role in assessing peanut accessions and cultivar development.

Pandey *et al*. (2012) worked on highly informative set of SSR markers in cultivated groundnut (*Arachis hypogaea* L.). A total of 4485 markers were used for screening using a set of 20 parental genotypes of 15 mapping populations. Although 3582 (79.9%) markers provided scorable amplification, only 1351 (37.3%) markers showed polymorphism. Polymorphism information content (PIC) value ranged from 0.10 (GM742) to 0.89 (S009) with an average of 0.31. Similarly, number of alleles

ranged from 2 to 14 with an average of 3.2 alleles. In general, the SSR markers based on dinucleotide repeats displayed higher PIC value and number of alleles. Based on these polymorphism features, 199 markers with >0.50 PIC values have been identified. Polymorphism features of these markers along with the primer sequences, for the first time, for a total of 946 SSR markers have been provided. It is anticipated that the identified set of highly informative markers, instead of starting from the random set of SSR markers, should be very useful to initiate molecular genetics and breeding studies in cultivated groundnut.

Goswami *et al*. (2013) constructed an experiment to detect molecular diversity using 35 SSRs in 12 mutant genotypes of groundnut. SSRs yielded an average of 3.57 polymorphic bands per primer. Polymorphism and PIC were 64.95% and 0.62%, respectively. The Cluster analysis found two major clusters that were separated by 61% Jaccard's similarity coefficient. A large number of genotypes were placed into a single cluster, demonstrating their shared lineage. AMOVA identified 15% of overall variance in kernel mass across 12 mutant genotypes and their parent. The K-W ANOVA revealed a significant relationship between five SSRs and kernel mass. TC3A12 and TC9H09 were related with large QTLs and accounted for 28 and 12% of phenotypic variance attributable to kernel mass, respectively. TC3A12 separated genotypes with higher kernel mass from genotypes with lower kernel mass by amplifying a band of about 450 bp from these two related primers. As a result, the link of the TC3A12 primer with a large QTL of kernel mass was verified further in genotypes with varied backgrounds. The TC3A12 primer distinguished between genotypes with higher kernel mass and genotypes with lower kernel mass by amplifying a 400 bp band among genotypes with higher kernel mass.

Amaravathi *et al*. (2014) studied set of 12 groundnut varieties released from Regional Agricultural Research Station, Tirupati and fingerprinted employing SSR markers. A total of 300 SSR were tested, with fifteen possible markers used for fingerprinting groundnut varieties. The SSR markers produced alleles ranging from two to seven, with an average of four per locus. The values for polymorphism information content (PIC) varied from 0 to 0.85. The genotypic data from all loci produced distinct SSR allelic fingerprints that aided in groundnut varietal identification. This study's core collection of highly informative primers, PM 377, TC1A02, TC5A06 and GM1489, has the capacity to identify the majority of groundnut varieties. SSR marker cluster analysis

separated 12 groundnut cultivars into two primary groups. Groundnut genotype fingerprinting provides information on phylogenetic relationships and aids groundnut breeders with varietal registration and intellectual property rights protection.

Roomi *et al*. (2014) evaluated genetic diversity of seventy pakistani accessions of *Arachis hypogaea* using thirty SSR primers. Out of thirty, fifteen primers produced polymorphic bands among the selected accessions. Throughout the accessions investigated, a total of forty polymorphic loci were found. The number of polymorphic loci discovered for each primer ranged from 2 to 4, with an average of 2.6 loci per primer. For each marker, the polymorphic index content (PIC) value was determined. The dendrogram was created using allelic data from fifteen SSR markers, which separated accession into six groups with 0.67 similarity coefficients. This study could aid in the conservation and breeding of groundnuts and other legumes.

Gaikpa *et al*. (2015) discovered molecular diversity among cultivated groundnut using SSR markers for further improvement. All 13 SSR markers were polymorphic, with PIC values over 0.50 in 76.9% of them. Genetic similarities between the 20 genotypes ranged from 60% to 90%, according to clustering analyses. As a result, there was molecular diversity in commercially grown groundnuts.

Oteng *et al*. (2015) characterized selected advanced breeding groundnut lines with different phenotypic attributes at the molecular level using simple sequence repeats (SSR) markers in Ghana. A total of 53 SSR markers were tested, and 25 were determined to be polymorphic, with a polymorphic information content (PIC) value of 0.57 on average. 67% of the 48 groundnut genotypes investigated had a very tight association (100% similarity) with one or more other genotypes. In fact, there were 14 instances when two to three genotypes within the same sub-cluster shared 100% resemblance, while having diverse phenotypic characteristics. The remaining 33% of groundnut genotypes were genetically distinct from one another and might thus provide as useful parental material for future research. Even when the amount of variation was modest, the SSR-based markers were found to be very selective in identifying differences across and among groundnut lines in this investigation. As a result, microsatellite-based markers are a powerful tool for analyzing genetic differences in farmed crops, particularly groundnut.

Patel *et al*. (2015) investigated 8 SSR primer out of ten SSR primers used generated a total of 21 bands, out of which 15 bands were polymorphic Two SSR primers did not amplify any fragment in PCR analysis. Only SSR primers generated 6 monomorphic bands that were equal to 68.75% polymorphism. The PIC values for SSRs ranged from 0.142 to 0.717 with an average of 0.445 for each primer. In present study, twelve groundnut genotypes were selected for their genetic diversity analysis and exploiting them in breeding program.

Wang *et al*. (2015) developed highly informative simple sequence repeat (SSR) markers and to assess the genetic diversity and population structure of peanut cultivars and breeding lines from different breeding programs in China, India and the US. For this study, 111 SSR markers were used, yielding a total of 472 alleles. Gene diversity and polymorphic information content (PIC) had mean values of 0.480 and 0.429, respectively. A country-by-country study indicated that alleles per locus were identical in three nations. The average gene diversity in the United States, China, and India was 0.363, 0.489 and 0.47, with PICs of 0.323, 0.43 and 0.412, respectively. The structure genetic analysis separated these peanut lines into two populations (P1, P2), which was compatible with the dendrogram based on genetic distance (G1, G2) and principal component analysis grouping. With a few admixtures, the categories were tied to peanut market kinds and geographic origin. Breeding programmes might utilize the findings to analyze the genetic variety of breeding materials in order to extend the genetic basis and for molecular genetics research.

Bhad *et al*. (2016) involved192 SSR markers for the development of *Arachis* genomic survey sequences. Seven polymorphic SSRs, 15 others genomic SSRs, 19 genic SSRs, and three STS markers were chosen from this set to detect genetic diversity among 44 groundnut genotypes. These polymorphic SSR markers amplified 155 bands (76 genomic and 79 genic), 128 of which were polymorphic (67 genomic and 61 genic). Allelic polymorphism was found 88.1% of genomic SSRs and 77.2% of genic SSRs. The markers polymorphic information content (PIC) ranged from 0.04 to 0.95. The pair-wise genetic similarity for genomic SSR markers ranged from 24.2 to 90.7% and 32.9 to 97.9% for genic SSR markers. Based on the pooled data from both genomic and genic SSRs, cluster analysis revealed a dendrogram that could distinguish the genotypes. Furthermore, the AMOVA analysis discovered 16.7% genetic variation due to seed size differences and 13.0% due to plant habit. Six markers were found to be

associated with plant habit and four markers with seed size based on locus-by-locus AMOVA and Kruskal-Wallis ANOVA and further confirmation by discriminant analysis and general linear model.

Rasam *et al*. (2017) carried out a study to assess the genetic diversity by using 10 simple sequence repeat (SSR) markers in groundnut. DNA was extracted from tender young leaf samples taken from eight different groundnut varieties. A protocol containing 0.090 g ml^{-1} glucose, 0.010 g ml^{-1} polyvinylpyrrolidone, 0.0040 g ml^{-1} sodium bisulphite, 0.0050 g ml^{-1} sodium dodecyl sulphate, and 50 µl ml^{-1} sarcosine resulted in high-quality DNA. A total of 88 scorable DNA fragments were generated, and 80 of them were found to be polymorphic between eight different varieties of groundnut. The minimum number of polymorphic fragments produced by pPGPseq2F05, pPGPseq8E12, pPGSseq13A10, pPGSseq16C06, pPGPseq2B10, PM384, PM137, PM003, and the maximum number of polymorphic fragments produced by pPGPSseq17F06 were found to be 16 (pPGPSseq17F06). The average % age of polymorphism revealed by SSR markers between the eight varieties was 90%. The overall similarity between all five varieties was calculated using SSR markers and ranged from 0.095 to 0.375. SSR marker analysis proved that SSR marker is a powerful tool for assessment of genetic diversity between groundnut varieties.

Nadaf *et al*. (2019) investigated groundnut genotypes at molecular level using simple sequence repeats (SSR). Twenty out of thirty SSR markers were found to be polymorphic, with an average polymorphism information content (PIC) value of 0.57. Fifty-seven percent of the 66 groundnut genotypes investigated exhibited a very tight association (80% similarity) with one or more other genotypes. The remaining 43% of groundnut genotypes were genetically distinct from one another and might thus provide as useful parental material for future research. SSRs were shown to be very selective in distinguishing differences between and among groundnut genotypes in this study, even when the amount of variation was modest.

Hong *et al*. (2021) analyzed morphological and molecular diversity of 101 peanut varieties from South China to identify distinctness among these varieties. Six morphological characteristics had no significant difference, whereas 11 morphological characteristics had a diversity index range of 0.25-0.51, with an average value of 0.39. Molecular characterization with 40 highly polymorphic simple sequence repeats

(SSRs) yielded a total of 167 alleles, with an average of 4.18 alleles per marker. These markers polymorphism information content (PIC) ranged from 0.79 to 0.26, with an average value of 0.55 per marker. The diversity analysis, which used morphological and genotyping data, classified all of the varieties into seven and six clusters, respectively, and varieties released by the same province tended to be grouped together. Mantel testing revealed that the correlations between the similarity coefficient matrices of morphological characteristics and SSR markers of different varieties were weak (r=0.347), implying that more SSR markers are required to achieve distinctness among these peanut varieties. Nonetheless, the combination of morphological characteristics and SSR markers will significantly improve distinctiveness identification accuracy.

Kalyani and Sasidharan (2021) investigated morphological and molecular characterization in different *Arachis* spp. The experiment was laid out in randomized complete block design with two replications and evaluated for 28 quantitative and 20 qualitative characters. The genetic distances between genotypes determined by analyzing 20 qualitative traits ranged from 1.00 between the pairs ICG1994 and AG2245 to 9.70 between the pairs ICG6813 and JB1180. The UPGMA method produced a dendrogram with four major clusters based on the average taxonomic distance matrix. The dendrogram was unable to establish a clear relationship between different botanical groups. However, a few genotypes from the Spanish bunch group tend to cluster together, indicating a shared phylogenetic relationship. This was also evident in the case of Virginia bunch cultures, which shared a phylogenetic relationship. A total of 1293 scorable bands with 251 alleles were found in a pooled SSR analysis of 50 groundnut genotypes using 23 SSR primers. The PIC value on average was 0.670. In this study, the average genetic similarity coefficient obtained by using SSR markers for different genotypes was 0.27.

Mofokeng *et al*. (2021) made study to assess the presence of genetic diversity among fifty-three groundnut genotypes of diverse origin using eleven agronomic and twenty SSR markers. The analysis of variance revealed that there were highly significant differences between genotypes for all phenotypic traits measured. Five major components accounted for 71% of total phenotypic variation. The polymorphic information content of the SSR loci ranged from 0.31 to 0.89, with a mean of 0.71. The heterozygosity values ranged from 0.03 to 1.00, with a mean of 0.57. The genotypes ranged in allelic diversity from 3 to 16, with an average of 8.1 alleles per locus. The

analysis of molecular variance (AMOVA) revealed that the majority of the variability (59%) was due to variation within individuals, with the remaining variation accounted for by variation among individuals within the population. Cluster analysis separated the genotypes into two distinct clusters, demonstrating that genotype discrimination was not dependent on the origin of the genotypes. The high gene flow observed among different geographic origins may contribute to the population's low differentiation. The SSR and phenotypic markers detected a wide genetic diversity and discriminated between groundnut genotypes. The two genetically distinct groups discovered in this study can be used as a source of novel genes and parental lines for transgressive segregation and further broadening of the crop's genetic base.

CHAPTER-III

MATERIALS AND METHODS

The present investigation on "**Genetic diversity analysis in groundnut (*Arachis hypogaea* L.) based on molecular markers and biochemical parameters**" was carried out at the Department of Biotechnology, Junagadh Agricultural University, Junagadh during the year 2021-22. Details of experimental materials used in the experiment, procedures followed and techniques adopted in the present investigation are described in this chapter.

3.1 EXPERIMENTAL MATERIALS

The investigation was carried out with 18 groundnut (*Arachis hypogaea* L.) varieties, which were procured from Main Oilseeds Research Station, Junagadh Agricultural University, Junagadh. The experiment material comprised of 18 genotypes of peanut which are listed in Table 3.1.

Table 3.1: List of groundnut varieties

Spreading type (Virginia runner-VR)	Semi-spreading type (Virginia bunch-VB)	Bunch type (Spanish bunch-SB)
1. GAUG-10	7. GG-21	13. GG-6
2. GG-11	8. GJG-22	14. GG-7
3. GG-16	9. GG-HPS-2	15. GJG-9
4. GJG-17	10. KDG-123	16. GJG-32
5. GG-41	11. KDG-128	17. JL-501
6. GJG-HPS-1	12. Kaushal	18. TG-37A

3.2 GLASSWARES AND POLYWARES

The glasswares and polywares used were of standard make such as Corning, Borosil or Schott Duran and plasticware (centrifuge tubes, microtips) from Tarsons or Axygen. All glasswares were scrubbed and washed thoroughly with detergent and then

rinsed with tap water followed by distilled water. Finally, it was dried in an oven before use. All the polywares were thoroughly cleaned as stated above and air-dried before use.

3.3 CHEMICALS

All the chemicals used in the experiment were of analytical grade from standard manufacturers like Sigma-Aldrich, E-Merck, Hi-media, Qualigenes and SISCO Research Lab. (SRL) etc. In case of fine chemicals, molecular biological grade was used, which were obtained from Merck bioscience, Bangalore.

3.4 EQUIPMENT

The different equipments were used in present study along with their details are described below (Table 3.2):

Table 3.2: List of equipment used in present study

Sr. No.	Name of equipment	Company
1	Weighing Balance	Citizen, CX 120
2	Hot water Bath	Nova
3	Gel Electrophoresis unit	Genetix
4	Gel Documentation machine	Upland and Gene Genius Bio Imaging System, SynGene
5	pH meter	Elico
6	Refrigerated Centrifuge	Plasto, Remi (C 24), Eltek
7	Thermal cycler-	Eppendorf, Applied Biosystems
8	Microcentrifuge	Eppendorf
9	-20°C Refrigerator	Operon
10	Hot air Oven	Nova
11	Pico drop	Qiagen
12	Microwave oven	IFB
13	Refrigerator	Samsung, Voltas

14	Water purification system	Millipore-Elix, India
15	Spectrophotometer	Thermo scientific evolution 201 UV visspectro.
16	Magnetic Stirrer with Hot plate	Remi Equipment Pvt. Ltd.
17	Micropipette of different size	Himedia, GeNei, Eppendorf

Table 3.3: Experimental details

1	**Location**	Department of Biotechnology, College of Agriculture, J.A.U., Junagadh	
2	**Year of experiment**	2021-22 (Laboratory trial)	
3	**Experiment details**		
3A	**Crop and Varieties**	Groundnut 18 varieties (6 from each spreading, semi-spreading, bunch type)	
	Spreading type (Virginia runner-VR)	Semi-spreading type (Virginia bunch-VB)	Bunch type (Spanish bunch-SB)
	1. GAUG-10	7. GG-21	13. GG-6
	2. GG-11	8. GJG-22	14. GG-7
	3. GG-16	9. GG-HPS-2	15. GJG-9
	4. GJG-17	10. KDG-123	16. GJG-32
	5. GG-41	11. KDG-128	17. JL-501
	6. GJG-HPS-1	12. Kaushal	18. TG-37A
3B	**Replication**	3 (Three)	
3C	**Experimental design**	CRD (Completely Randomized Design) for biochemical parameters	

4	**Observation recorded**			
4A	**Biochemical parameters**	1)	Moisture content	A.O.A.C. (2005b)
		2)	Oil Content	A.O.A.C. (2005b)
		3)	Total Carbohydrate	Hedge and Hofreiter (1960)
		4)	Total Protein	Kjeldahl Method, A.O.A.C. (2005a)
		5)	True Protein	Lowry *et al*. (1951)
		6)	Total Soluble Sugar	Dubois *et al*. (1956)
		7)	Reducing Sugar	Somogyi (1952)
		8)	Non-Reducing Sugar	By difference (total soluble sugar - reducing sugar content)
		9)	Ash	A.O.A.C. (2005c)
4B	**Molecular Characterization**	1)	DNA extraction and purification	Wanjira *et al*. (2020)
		2)	RAPD analysis	Yaikhom *et al*. (2019)
		3)	ISSR analysis	Sharma *et al*. (2017)
		4)	SSR analysis	Shoba *et al*. (2010)

3.5 BIOCHEMICAL PARAMETERS

Following proximate parameters were analysed from mature seed:

3.5.1. Moisture content

The moisture content of groundnut samples was determined using the Association of Official Analytical Chemists (AOAC) method (2005). The moisture content of the samples was determined by weighing 5 g of sample into a petri plate and

dried them at 90°C for 5 hours in a hot air oven. When the Petri dishes reached to room temperature, they were immediately transferred to a desiccator and weighed. The calculation of moisture content was done by using following formula.

$$\text{Moisture (\%)} = \frac{\text{Weight of fresh sample (g) - Weight of oven dried sample (g)}}{\text{Weight of fresh sample (g)}} \times 100$$

3.5.2. Oil Content

Oil content was determined by Soxhlet extraction (AOAC, 2005). Dried groundnut seed crush in mortar and pestle. The sample weighed accurately 5 g and put into thimbles filled with 150 ml hexane. Cotton wool was used to plug these thimbles. The Soxhlet apparatus was then assembled and left to reflux for 4 hours. The thimble was carefully removed. The extraction flask is allowed to cool before being dismantled. Evaporate the hexane remaining in the water bath until there is no odour of hexane. The moisture on the outside of the flask is removed and weighed. Per cent oil content was calculated using the following formula.

$$\text{Oil (\%)} = \frac{\text{Weight of oil flask after extraction (g) - Weight of empty flask (g)}}{\text{Weight of the dried sample (g)}} \times 100$$

3.5.3. Total Carbohydrate

Anthrone method was used to analyze total carbohydrate spectrophotometrically (Hedge and Hofreiter, 1962). Weighed 0.5 g of the grinded sample into a flask. It was hydrolysed in a boiling water bath for 3 hours with 5 ml of 2 N HCL and then cooled to room temperature. Used solid sodium carbonate to neutralize it. Centrifuged and supernatant was collected into 50 ml volumetric flask. An aliquot (0.1 ml) was placed in a test tube and made to a volume of 1 ml with distilled water. Dissolve 100 mg glucose in 100 ml of water to make a standard glucose + stock solution. Ten ml of stock diluted to 100 ml with distilled water as a working standard. Prepared the set of standards by taking 0 ml, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml and 1 ml of the working standard, with the '0' serving as a blank. The volume made up to 1 ml in all the tubes including the sample tubes by adding distilled water. Then, added 4 ml of fresh anthrone reagent (Dissolve 200 mg anthrone in 100 ml of ice-cold 95 % H_2SO_4). Heat for ten minutes in a boiling water bath. Cool rapidly and read the green to dark green color at 630 nm. The amount of carbohydrate present in the sample was calculated in % using following formula.

$$\text{Total carbohydrate (\%)} = \frac{\text{Graph factor x Sample reading x Total volume (ml) x 100 x } 10^{-6}}{\text{Sample aliquot (ml) x Weight of sample (g)}}$$

3.5.4. Total Protein

The total protein content of the samples was determined using the micro Kjeldahl's method, as defined in AOAC (2005), which included protein digestion and distillation as stated below.

3.5.4.1 Digestion

The samples were weighed in triplicate and placed in digestion flasks at 0.5 g each. Each flask received a few anti-bumps granules and approximately 3 g of copper digestion mixture (96% potassium sulphate, 3.5% copper sulphate and 0.5% mercury oxide). The digestion process was then started by adding 10 ml of concentrated sulphuric acids to each flask and heating it on a heating mantle. The flask was allowed to cool after digestion was completed until a clear solution was obtained. The digest was then filtered and diluted to a volume of 10 ml with distilled water. During the distillation step, 10 ml of the diluted digest was pipetted into round-bottom flasks.

3.5.4.2 Distillation

A round-bottom flask was set on a heating mantle and connected, using a Liebig condenser, to a beaker (receiver flask) containing 20 ml of 4% boric acid with screened methyl red indicator. The condenser was submerged in the boric acid by the use of a Buchner funnel. Exactly 30 ml of 40% sodium hydroxide was then injected into the flask and distillation of the ammonia formed commenced by heating the flask. The distillation was continued until the boric acid solution completely changed from purple to greenish yellow. The boric acid mixture (containing the ammonium borate complex formed) was then titrated with 0.05 N H_2SO_4 to colorless end point. The total organic nitrogen was then calculated using the formula:

After titration, the % nitrogen was calculated using the following formula:

$$\% \text{ Nitrogen} = (R\text{-}B) \times \text{N of acid} \times 0.014 \times 100 / W$$

Then, percentage crude protein in the sample was calculated from the

$$\% \text{ Nitrogen as } \% \text{ Total protein} = \% \text{ N x } 6.25$$

Where, R = Required hydrochloric acid for sample to titrate sample; B = Volume (ml) of acid required to titrate the blank; N of acid= Normality of acid; W=Weight of sample (g).

3.5.5 True Protein

The Folin-Lowry method was used to obtain true protein (Lowry *et al*., 1951) The 0.5 gm grinded groundnut seed was first extracted with 10 ml 0.1 N NaOH. A suitable aliquot of 0.1 ml was taken, and a total volume of 3 ml was made with distilled water and 5 ml of reagent C (Reagent A: 2% Sodium Carbonate in 0.1 N Sodium Hydroxide; Reagent B: 0.5 % copper sulphate in 1% sodium potassium tartrate prepared fresh by combining 50 ml of reagent A with 1 ml of reagent B in a 50:1 ratio) was added and thoroughly mixed. After 10 minutes, 0.5 ml of reagent D (Reagent D: Folin-Ciocalteau reagent diluted with distilled water in a 1:1 ratio) was added, thoroughly mixed and left at room temperature for 30 minutes to develop the color. For working standard series, the same protocol was used. At 660 nm, the absorbance was measured and expressed as a percentage. The protein content was calculated by using Bovine serum albumin as standard. The amount of protein in the sample was determined using following formula.

$$\text{True protein}(\%) = \frac{\text{Graph factor x Sample reading x Total volume (ml) x 100 x } 10^{-6}}{\text{Sample aliquot (ml) x Weight of sample (g)}}$$

3.5.6 Total Soluble Sugar

Total Sugar content was estimated by Phenol Sulfuric acid method with some modifications (Dubois *et al*., 1956). In hot acidic medium glucose is dehydrated to hydroxymethyl furfural. This forms a green colored product with phenol and has absorption maximum at 490 nm. The sample weight 0.2 gm taken and homogenized in mortar and pestle with 5 ml of 80% methanol Then, pipette out 0.2 ml of the sample aliquot in three separate test tubes. Final volume made up in each tube to 1 ml with distilled water. Set a blank with 1 ml of distilled water. Simultaneously, 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard were taken into a series of test tube. Added 1 ml of 5% redistilled phenol solution to each tube and incubated at room temperature for 10 min. Then, added 5 ml of 96% sulphuric acid to each tube and shake well. After 10 min shaking incubated at room temperature for 30 min. The color intensity read at 490 nm. The amount of total soluble sugar in the sample was determined using following formula.

$$\text{Total soluble sugar}(\%) = \frac{\text{Graph factor x Sample reading x Total volume (ml) x 100 x } 10^{-6}}{\text{Sample aliquot (ml) x Weight of sample (g)}}$$

3.5.7 Reducing Sugar

Nelson-Somogyi's method was used to calculate the reducing sugar (Somogyi, 1952). The sample weight was taken 0.1 gm, and it was homogenized in a mortar and pestle with 80% methanol before being centrifuged at 3000 rpm for 10 minutes. The extraction was repeated four times, and the supernatant was collected into a 25 ml volumetric flask and diluted with 80 percent methanol to make a volume of 25 ml. Pipette 0.5 ml aliquots from each sample into separate test tubes. Simultaneously 0.2, 0.4, 0.6, 0.8 and 1 ml of the working standard were taken into a series of test tube. The volume made up to 1 ml in both sample and standard tubes with distilled water. To create a blank, pipette out 1 ml of distilled water in a separate tube. Then 1 ml of alkaline copper reagent (4 gm copper sulphate, 24 gm sodium carbonate, 12 gm sodium potassium tartarate, 16 gm sodium bicarbonate and 180 gm anhydrous sodium sulphate dissolved in 1000 ml of DW) was added. Place the tubes in boiling water bath for 10 min, then add 1 ml arsenomolybdate reagent (50 gm Ammonium molybdate, 42 ml concentrated sulphuric acid, 6 gm disodium hydrogen arsenate well mixed in 1000 ml DW) into each test tube. DW was used to make a total volume of 10 ml. The absorbance was then measured at 620 nm. The amount of reducing sugar present in the sample was calculated in % using appropriate formula.

$$\text{Reducing sugar(\%)} = \frac{\text{Graph factor x Sample reading x Total volume (ml) x 100 x } 10^{-6}}{\text{Sample aliquot (ml) x Weight of sample (g)}}$$

3.5.8 Non-Reducing Sugar

The non-reducing sugar was calculated by difference of total soluble sugar and reducing sugar content and expressed as %.

3.5.9. Ash

Ash is an inorganic residue remaining after the material has been completely burnt at a temperature of 600 °C in a muffle furnace. It is the aggregate of all non-volatile inorganic elements. About 0.5 g of finely ground dried sample was weighed into a porcelain crucible and incinerated at 600 °C for 5 hr in an ashing muffle furnace until ash was obtained. After cooling, sample was weighed as quickly as possible to prevent moisture absorption. The % ash content in the groundnut sample was calculated as follows:

$$\text{Ash (\%)} = \frac{\text{Weight of ashed sample (g)}}{\text{Weight of the sample (g)}} \times 100$$

3.6 MOLECULAR CHARACTERIZATION

3.6.1 DNA Extraction

Genomic DNA was isolated from germinated seedlings of groundnut varieties by method as described by Wanjira *et al*. (2020).

3.6.1.1 Preparation of stock solution for reagents and buffer for DNA extraction

The reagent for DNA extraction were prepared as per Wanjira *et al*. (2020). The composition and procedure for preparation of various stock solution and buffers are given in Table 3.4

Table 3.4: Preparation of stock solutions for DNA extraction and agarose gel electrophoresis

Sr. No.	Chemicals /Reagents	Procedure
1	80 % Ethanol (100 ml)	80 ml of ethanol from 100% pure was taken and 20 ml of D/W was added, mixed well and dispensed to reagent bottle and stored at room temperature.
2	Chloroform: Isoamyl alcohol (24:1) (100 ml)	96 ml of chloroform and 4 ml of isoamyl alcohol were mixed and stored in reagent bottle at room temperature.
3	Ethidium Bromide (10 $mg.ml^{-1}$)	100 mg Ethidium Bromide was added to 10.0 ml of distilled water and it was kept on magnetic stirrer to ensure that the dye is dissolved completely. It was transferred into eppendorf tube and stored at 4°C.
4	1X TE buffer (100 ml) pH 8.0	121 mg of Tris-Base, 37 mg EDTA were taken and dissolved in D/W and volume was adjusted to 100 ml. The solution was autoclaved and stored at room temperature.
5	TBE buffer 5X (1 litre) pH 8.0	113 g of tris-base, 55 g of boric acid and 9.3 g EDTA were taken and dissolved in distilled water. The final volume was adjusted to 1 liter by D/W and the pH was adjusted to 8.0.

6	6X- Gel loading dye	4.0 g sucrose and 0.025 g BPB were dissolved in double distilled water and made up the final volume up to 10 ml.
7	5M NaCl (100 ml)	29.2 g NaCl was taken in to beaker 50 ml of D/W was added and mixed well. When the salts get completely dissolved, the final volume was adjusted to 100 ml. It was dispensed to reagent bottle and sterilized by autoclaving.
8	1M Tris HCl (100 ml) pH 8.0	12.1 gm Tris base was dissolved in 80 ml D/W. The pH was adjusted to 8.0 by adding concentrated HCl. A total volume was adjusted to 100 ml. It was dispensed to reagent bottle and sterilized by autoclaving.
9	0.5M EDTA (100 ml) pH 8.0	18.6 gm EDTA di Sodium salt was dissolved in 80 ml D/W. The pH was adjusted to 8.0 by adding NaOH pellets. A total volume was adjusted to 100 ml. It was dispensed to reagent bottle and sterilized by autoclaving.
10	10% CTAB, 100ml	10 gm of CTAB powder was taken and it was added into boiling water to dissolve completely and the final volume was made up to 100 ml.
11	4% PVP, 100 ml	4 gm PVP was taken into beaker and 96 ml of distilled water was added, mixed well and dispensed to reagent bottle and stored at 4°C.
12	RNase (10 mg/ml)	Weigh 100 mg of RNase and dissolve in 0.01 M sodium acetate (pH 5.2). Heat to 100°C for 15 minutes and allow cooling to room temperature. Adjust pH by 1 M TrisCl (pH 7.4). Dispense into aliquots and store at -20°C.

Table 3.5: Preparation of extraction buffer for DNA extraction

Stock solution	**Final concentration**	**Amount per 100ml**
CTAB	2%	20 ml

NaCl	5 M	28 ml
Tris (pH-8)	1 M	20 ml
EDTA (pH-8)	0.5 M	4 ml
Urea	0.5%	0.5 g
PVP	4%	4 ml
Distilled Water		24 ml
Total		**100 ml**

3.6.1.2. Protocol for isolation of genomic DNA

1. One gm of leaf tissue was ground in liquid N_2 with the help of mortar and pestle.
2. Pre-warmed (1.5 ml, 65^0C) DNA extraction buffer (2X CTAB extraction buffer) was added to the homogenized leaf material.
3. Homogenized material was transferred in to capped polypropylene tubes and incubated at 65^0C for 45 min in water bath with gentle swirling.
4. This was spun at 10,000 rpm for 15 minutes at room temperature (25^0C).
5. One ml aqueous phase was transferred to another fresh tube and 1 ml Chloroform: Isoamyl alcohol (24:1) was added and mixed by inversion for 15 minutes to ensure emulsification of the phase and spun at 10,000 rpm for 10 minutes at room temperature (25^0C).
6. Aqueous phase was transferred to another fresh tube.
7. The above steps 5 to 6 repeated twice.
8. Equal volume of ice-cold iso-propanol was added to aqueous phase to precipitate DNA.
9. The tube were centrifuged at 10,000 rpm, 4°C for 15 min and the supernatant was discarded.
10. After removing supernatant, 1.5 ml of 80% alcohol was added in pellet and was kept for 10 minutes with gentle agitation.
11. Then, it was centrifuged at 10,000 rpm for 15 minutes at 4^0C.
12. The tubes were inverted and drained on a paper towel. The pellet was air dried for 45 minutes.
13. Each pellet was re-dissolved in 100µl of TE buffer by keeping over night at room temperature without agitation.

3.6.1.3 Purification of DNA

To get RNA free DNA sample, the purification was carried out as follows.

1. One µl of RNase was added to 100 µl of crude DNA preparation.
2. It was mixed thoroughly and incubated at room temperature for 15 minutes.
3. Ten µl of 3M sodium acetate was added and mixed thoroughly for 10 minutes till an emulsion was formed.
4. Spun at 10,000 rpm for 10 minutes at 4^0C.
5. Supernatant was taken, avoiding the whitish layer at interface.
6. The DNA was re-precipitated by adding double the quantity of absolute ethanol (isopropenol). To pellet the DNA, the tubes were centrifuged at 5000 rpm for 5 minutes at 4^0C.
7. The pellet was washed with 500µl 80% ethanol and air dried for 30 minutes.
8. The DNA was re-dissolved in 100 µl of TE buffer for further use.

3.6.1.4 Estimation of quality and quantity of DNA

In order to perform PCR based analysis, the DNA concentration was determined by picodrop (Qiagen). The 0.2 µl sample mounted in QIAxpert Slide-40 for measurement of quality at A260/A280 ratio which was found between 1.50 to 2.09 among 18 genotypes/varieties and the quantity was directly displayed as ng.µl^{-1} that is given in Table 3.6. The concentration of DNA was adjusted 50 ng.µ$l^{-1}$for further work.

Table 3.6: Purity and concentration of groundnut DNA samples

Sr. No.	Varieties	Absorbance A_{260}/A_{280} ratio	Concentration ng.µl^{-1}
1	GAUG-10	1.40	424.3
2	GG-11	2.03	944.5
3	GG-16	1.50	454.6
4	GJG-17	1.99	1189.2
5	GG-41	2.02	158.7
6	GJG-HPS-1	2.03	453.0
7	GG-21	2.09	829.3
8	GJG-22	2.03	301.3
9	GG-HPS-2	2.03	379.2

10	KDG-123	1.99	587.4
11	KDG-128	1.97	290.5
12	Kaushal	2.04	401.8
13	GG-6	2.00	413.0
14	GG-7	2.00	489.3
15	GJG-9	2.00	380.2
16	GJG-32	2.06	298.1
17	JL-501	2.04	438.7
18	TG-37A	2.03	386.8

3.6.2 Agarose Gel Electrophoresis

To measure the integrity of DNA, agarose gel electrophoresis was done.

3.6.2.1 Chemicals used for agarose gel electrophoresis

a) Agarose (low EEO type) (Himedia, Bangalore)

b) 5 X Tris Borate EDTA (TBE) buffer pH 8.0

c) Gel loading dye (6X)

d) Ethidium bromide (10 mg.ml^{-1})

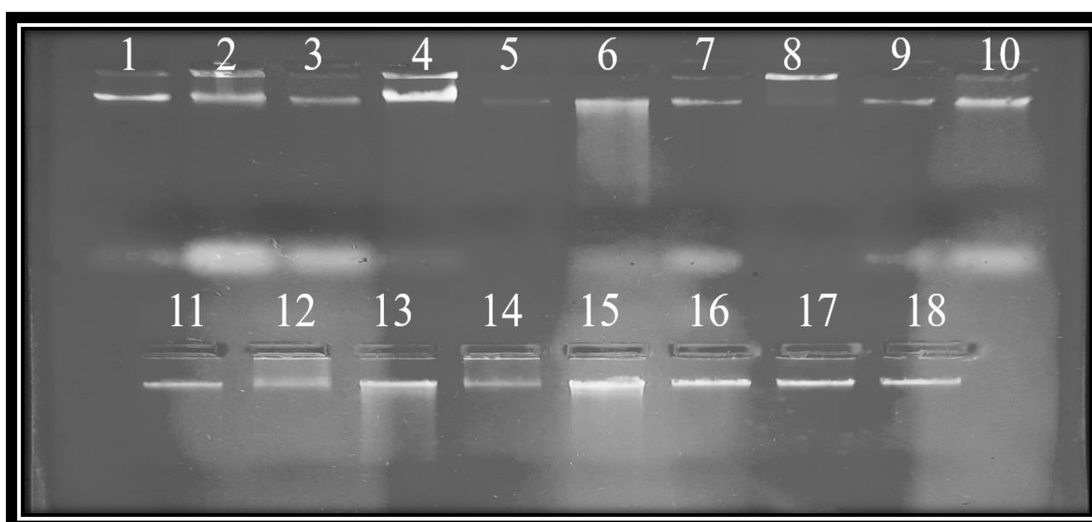


Fig. 3.1: Electrophoretic banding pattern of genomic DNA of groundnut on 0.8 % Agarose gel (1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

3.6.2.2 Preparation of gel

1. Agarose gel of 0.8 % was prepared (dissolve 0.8 g agarose in 100 ml 1X TBE and 5.0 µl EtBr from 10 mg/ml stock).
2. About 5µl of DNA + 2 µl of 1 X gel loading dye were loaded in each well.
3. The gel was run at 100 V for 30 minutes and DNA bands were visualized under UV transilluminator (254nm) which was photographed using Gel Documentation System.
4. Presence of single compact band on agarose gel indicated integrity of isolated DNA.

3.7 MOLECULAR MARKERS ANALYSIS OF GROUNDNUT

For fingerprinting groundnut entries, various molecular marker techniques such as Randomoly Amplified Polymorphic DNA (RAPD), Simple Sequence Repeat (SSR), and Inter Simple Sequence Repeat (ISSR) were used. Primers required for the above techniques were synthesized from Merck bioscience, Bangalore.

All primers for RAPD, ISSR and SSR were diluted by adding equal amount of deionized sterile D/W equal to its concentration. e.g. If the concentration of RAPD primer is 89 nM then adding 89 µl of D/W which finally made a concentration of 1 nM.µl^{-1}=1000 pM.µl^{-1}. This was kept as a stock solution of primer. By taking 5 µl of stock (1000 pMoles.µl^{-1}) and 195 µl of deionized sterile D/W gave final concentration of 25pM.µl^{-1}. This working solution is used for PCR amplification for various molecular techniques.

3.7.1 Randomly Amplified Polymorphic DNA (RAPD)

The PCR process for RAPD was performed according to method given by Yaikhom *et al*. (2019) with some modifications. The RAPD assays were performed using random 10-mer oligonucleotide primers (Table 3.7).

3.7.1.1 PCR Reagents

The reagents used for PCR amplification of DNA are as under:

a. 10X PCR buffer [Tris (pH 9.0), KCl, 15 mM $MgCl_2$, Gelatin] (Merck bioscience, Bangalore)

b. Taq DNA polymerase (Merck bioscience, Bangalore)

c. dNTP mix (Merck bioscience, Bangalore)

d. Primer (25 pMoles.µl^{-1})

Table 3.7: List of RAPD primers

Sr. No.	RAPD Primer	Sequence 5'- 3'	GC (%)	Tm (°C)
1	OPA-16	5'-AGCCAGCGAA-3'	60	25
2	OPC-05	5'-GATGACCGCC-3'	70	27
3	OPD-05	5'-TGAGCGGACA-3'	60	25
4	OPL-01	5'-GGCATGACCT-3'	60	32
5	OPL-04	5'-GACTGCACAC-3'	60	32
6	OPN-04	5'-GACCGACCCA-3'	70	27
7	OPN-10	5'-ACAACTGGGG-3'	60	25
8	OPN-13	5'-AGCGTCACTC-3'	60	25
9	OPN-16	5'-AAGCGACCTG-3'	60	32
10	OPQ-14	5'-GGACGCTTCA-3'	60	25
11	OPZ-10	5'-CCGACAAACC-3'	60	25
12	OPZ-11	5'-CTCAGTCGCA-3'	60	32
13	OPZ-19	5'-GTGCGAGCAA-3'	60	32
14	CMN-A01	5'-AGCAGCGCCTCA-3'	66.7	40
15	CMN-A11	5'-ACTGACCTAGTT-3'	41.7	34
16	CMN-A24	5'-GACGGTTCAAGC-3'	58.3	38
17	CMN-A26	5'-GGTGAGGATTCA-3'	50	36
18	CMN-A37	5'-AGCGCGGCAAAA-3'	58.3	38
19	CMN-A40	5'-GCGGAGGAACCA-3'	66.7	40
20	CMN-A44	5'-AAGGACACAACA-3'	41.7	34
21	CMN-A55	5'-TACGCCGGAATA-3'	50	36

3.7.1.2 PCR protocol

The master mixture was prepared in a microfuge tube in which the buffer was added first followed by sterile D/W, primer, dNTPs mix followed by Taq DNA polymerase. At the last DNA was added in each tube separately (Table 3.8).

Table 3.8: Preparation of reaction mixture for RAPD

Sr. No.	Reagent	Quantity
1	PCR buffer (10X) (without $MgCl_2$)	1.5 µl
2	Taq polymerase (3 U/µl)	0.15 µl
3	dNTPs mix (2.5 mM each)	1.2 µl
4	Primer (25 pMoles/µl)	1.2 µl

5	Template DNA (50 ng/µl)	1.2 µl
6	Millipore sterile distilled water	9.75 µl
	Total	**15.0µl**

The reagents were mixed gently by tapping against the tube. The tubes were then placed in the Thermal Cycler for amplification. The PCR condition for thermal cycler is given in Table 3.9.

Table 3.9: PCR conditions for RAPD

Sr. No	Steps	Temperature (ºC)	Duration
1	Initial Denaturation	94	5.0 min
2	Denaturation	94	1.0 min
3	Annealing	37	1.0 min
4	Extension	72	2.0 min
Repeat the steps 2 to 4 for 35 times			
5	Final extension	72	7.0 min
6	Hold	4	--

All the PCR reactions were carried out in 0.2 ml capacity thin walled PCR tubes. As per the above cocktail, Millipore sterilized water was added first followed by addition of PCR master mix (Bangalore genei Pvt Ltd.), primer in sequence and finally the template DNA. The reagents were mixed gently by tapping against the tube followed by a short spinning (~3,000 rpm for 30 seconds). The tubes were then placed in the Thermal Cycler for cyclic amplification.

3.7.1.3 Electrophoresis of amplified product

The amplified products of RAPD were analyzed using 1.2 % agarose gel in TBE buffer. Three µl of 6 X loading dye was added to each PCR tube containing amplified product. Loaded 12-15 µl of the sample to each well using micropipette. Three µl of the 100 bp-1kb DNA ladder was loaded in the first well. Then electrophoresis was conducted at 100 V current (constant) for 45 min to separate the amplified bands. The separated bands were visualized under UV trans-illuminator photographed using Gel Documentation System and molecular weight was calculated using AlphaEaseFC 4.0 software.

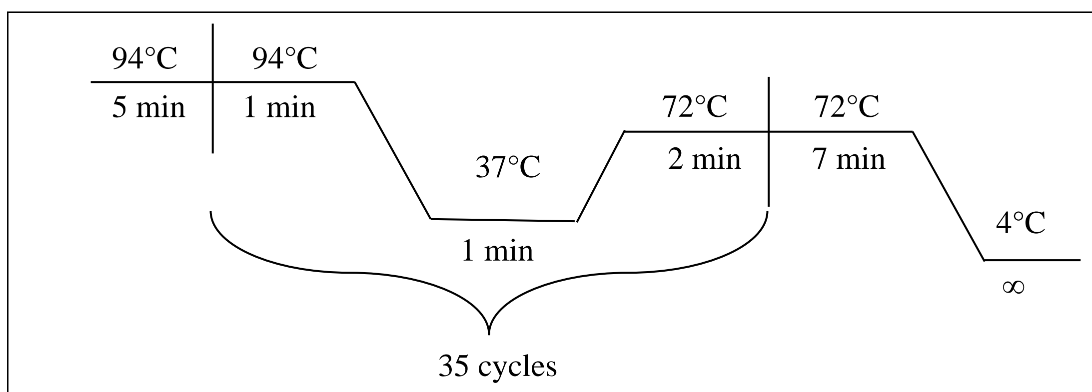


Fig. 3.2: Steps in RAPD PCR amplification

3.7.2 Inter Simple Sequence Repeats (ISSR)

The PCR reactions for ISSR were carried according to method given by Sharma *et al*. (2017) with required modifications. The genomic DNA was amplified using ISSR primer series (Table 3.10).

Table 3.10: List of ISSR primers

Sr. No.	ISSR Primer	Sequence (5'→3')	GC (%)	Tm (°C)
1	UBC-801	5'-$(AGC)_6$-3'	67.0	68.6
2	UBC-802	5'-$(AG)_8$G-3'	53.0	46.6
3	UBC-803	5'-$(AT)_8$C-3'	5.88	23.8
4	UBC-805	5'-$(AG)_8$T-3'	47.0	42.4
5	UBC-806	5'-$(AG)_8$G-3'	52.9	46.5
6	UBC-810	5'-$(GA)_8$T-3'	47.1	50.4
7	UBC-811	5'-$(TC)_8$C-3'	47.4	52.9
8	UBC-812	5'-$(AC)_7$T-3'	47.0	49.2
9	UBC-813	5'-$(AC)_8$C-3'	53.0	53.3
10	UBC-848	5'-$(CA)_8$RG-3'	50	55.5
11	UBC-852	5'-$(TC)_8$RA-3'	44.44	44.5
12	UBC-857	5'-$(AC)_8$YG-3'	60	53.7
13	UBC-862	5'-$(AGC)_6$-3'	66.67	68.6

3.7.2.1 PCR Protocol

The mastermix was prepared in a microfuge tube in which the buffer was added first followed by sterile water, Primer, dNTPs mix followed by Taq DNA polymerase (Table 3.11). At the last DNA was added in each tube separately.

Table 3.11: Preparation of reaction mixture for ISSR

Sr. No.	Reagent	Quantity
1	PCR buffer (10X) (without $MgCl_2$)	1.5 µl
2	Taq polymerase (3 U/µl)	0.15 µl
3	dNTPs mix (2.5 mM each)	1.2 µl
4	Primer (25 pMoles/µl)	1.2 µl
5	Template DNA (50 ng/µl)	1.2 µl
6	Millipore sterile distilled water	9.75 µl
	Total	**15.0µl**

The reagents were mixed gently by tapping against the tube. The tubes were then placed in the Thermal Cycler for amplification. The PCR condition for thermal cycler is given in Table 3.12.

Table 3.12: PCR conditions for ISSR

Sr. No.	Step	Temperature (ºC)	Duration
1	Initial Denaturation	94	4.0 min
2	Denaturation	94	1.0 min
3	Annealing	Tm±2	1.0 min
4	Extension	72	2.0 min
Repeated the steps 2 to 4 for 40 times			
5	Final Extension	72	8.0 min
6	Hold	4	∞

All the PCR reactions were carried out in 0.2 ml capacity thin walled PCR tubes. As per the above cocktail, Millipore sterilized water was added first followed by addition of PCR master mix (Bangalore genei Pvt Ltd.), primer in sequence and finally the template DNA. The reagents were mixed gently by tapping against the tube followed by a short spinning (~3,000 rpm for 30 seconds). The tubes were then placed in the Thermal Cycler for cyclic amplification.

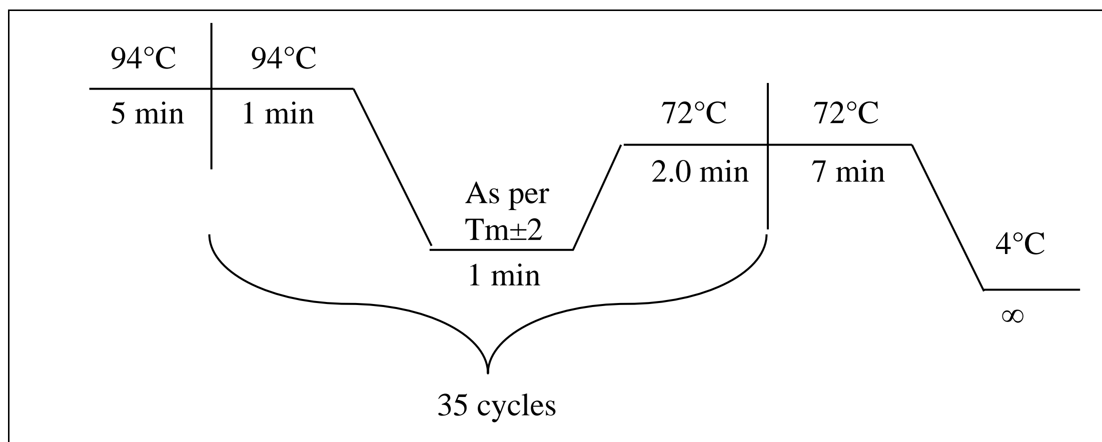


Fig. 3.3: Steps in ISSR PCR amplification

3.7.2.2 Electrophoresis of amplified product

PCR products were subjected to electrophoresis with marker DNA of known molecular weight in 1.5 % agarose gel. After electrophoresis, the gel was carefully taken out of the casting tray and photographed in SynGene gel documentation system.

3.7.3 Simple Sequence Repeat (SSR)

The PCR reactions for SSR were prepared according to method given by Shoba *et al*. (2010) with some modifications for PCR conditions. The genomic DNA was amplified using SSR primer series (Table 3.13).

Table 3.13: List of SSR primers

Sr. no.	Primer name	Sequence (5’ to 3’)	GC (%)	Tm (°C)
1	JAUGP 100	F: ATGTCCATCAAATGCCTCAAC	53.5	42.9
		R: TACGCCTCAGATTTGTTTCCA	54.2	42.9
2	JAUGP 101	F: CCACCACATTACAACTTACGAAC	54.0	43.5
		R: GTGCATTCAACGCCACAA	54.3	50.0
3.	JAUGP 103	F: TTTTCCACTTCCCCTCTTGTT	54.3	42.9
		R: AATCATAACCCCACCCCTTC	54.5	50.0
4	JAUGP 106	F: CGAGGGAATTGCTGTTTGTG	54.5	50.0
		R: TCACATTATTGAGCTTCATGGTT	52.6	34.8
5	JAUGP 145	F: GAAGGATGGCTCATTGTCGT	55.0	50.0

		R: TAAAAGAAGGGGGTAGAGTTG	51.6	42.9
6	JAUGP 175	F: CAGCAGCAACAACAACAACAA	54.9	42.9
		R: CTTTCGTCCCGATACTCCAA	54.2	50.0
7	JAUGP 182	F: GGGTTCTATTGTTGGGATTGG	53.5	47.6
		R: AAGAACGGTGGTGGATTATTG	52.9	42.9
8	JAUGP 197	F: CATTCCTTCCTTCAAAAACACA	51.6	36.4
		R: GAGCCAAGAATAAGAAGAGTCCA	54.3	43.5
9	JAUGP 253	F: CATTCCAATCTTTCCCTCCAC	53.5	47.6
		R: AGGCCAAGAAGGAGTTGTTT	54.5	45.0
10	JAUGP 256	F: CGCACAGCTATCACAATCACA	55.5	47.6
		R: TGAGCGAGGAAATTATCAAGG	52.4	42.9
11	JAUGP 277	F: GGCAGAAGCAAACGGAAC	54.6	55.6
		R: AGAAGACGACAACGAAGACCA	56.0	47.6
12	JAUGP 350	F: GGGGGAAAGAAGAAAGAAGAAA	52.8	40.9
		R: CGTTCACCAGAAACCCCTAAA	55.0	47.6
13	PM-36	F: ACTCGCCATAGCCAACAAAC	50.0	57.3
		R: CATTCCCACAACTCCCACAT	50.0	57.3
14	PM-188	F: GGGCTTCACTGCTTTTGATT	45.0	55.3
		R: TGCGACTTCTGAGAGGACAA	50.0	57.3
15	PM-238	F: CTCTCCTCTGCTCTGCACTG	60.0	61.4
		R: ACAAGAACATGGGGATGAAGA	42.9	55.9
16	PM-325	F: CCTAACAAGGACGGGTGAAC	55.0	59.4
		R: CAGAGGCCTCACTTTCCTTC	55.0	59.4
17	TC3H07	F: CAATGGGAGGCAAATCAAGT	45.0	55.3
		R: GCCAAATGGTTCCTTCTCAA	45.0	55.3
18	TC1E05	F: GAAGGATAAGCAATCGTCCA	45	55.3
		R: GGATGGGTTGAACATTTGG	45	55.3

3.7.3.1 PCR protocol

The master mixture was prepared in a micro centrifuge tube in which the buffer was added first followed by sterile water, Primer, dNTPs mix followed by Taq DNA polymerase (Table 3.14). At the last DNA was added in each tube separately.

Table 3.14: Preparation of reaction mixture for SSR

Sr. No.	Reagent	Quantity
1	PCR buffer (10X)	1.5 µl
2	Taq polymerase (3 U.μl^{-1})	0.15µl
3	dNTPs mix (2.5 mM each)	1.2 µl
4	Primer (25 pMoles.μl^{-1})	1.2 µl
5	Template DNA (50 ng.μl^{-1})	1.2 µl
6	Millipore sterile D/W	9.75 µl
Total		**15 µl**

The reagents were mixed gently by tapping against the tube. The tubes were then placed in the Veriti 96-well Thermal Cycler for amplification. The PCR condition for thermal cycler is given in Table 3.15.

Table 3.15: PCR conditions for SSR

Sr. No	Steps	Temperature (°C)	Duration
1	Initial Denaturation	94	4.0 min
2	Denaturation	94	45 sec
3	Annealing	52	45 sec
4	Extension	72	1 min
Repeat the steps 2 to 4 for 35 times			
5	Final extension	72	7 min
6	Hold	4	-

3.7.3.2 Electrophoresis of amplified product

PCR products were subjected to electrophoresis with marker DNA of known molecular weight in 1.5 % agarose gel. After electrophoresis, the gel was carefully taken out of the casting tray and photographed in SynGene gel documentation system.

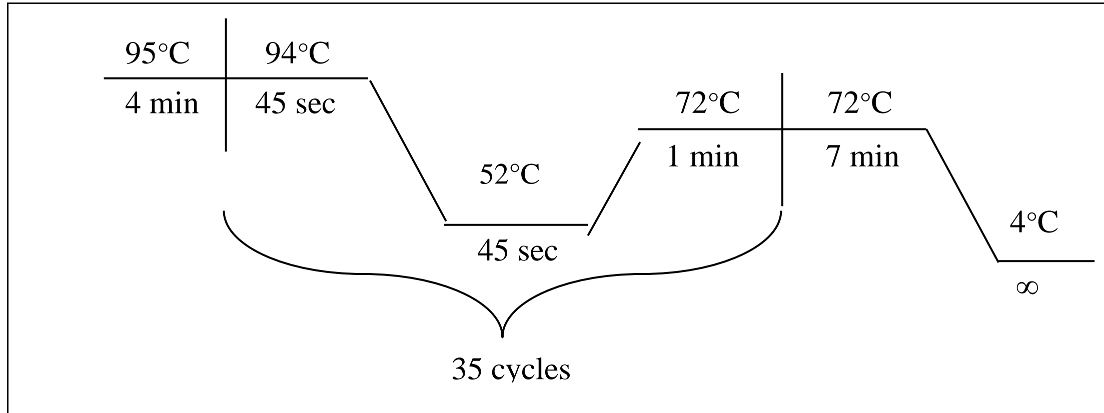


Fig. 3.4: Steps in SSR PCR amplification

3.8 STATISTICAL ANALYSIS FOR MOLECULAR PARAMETERS

3.8.1 Polymorphic Information Content (PIC) Calculation

PIC for RAPD, ISSR and SSR was calculated on the basis of allele frequency (Anderson *et al.,* 1993).

$$PIC_i = 1 - \sum_{j=1} P_{ij}^2$$

Where P_{ij} is the frequency of jth allele for marker i, and summation extends over n alleles.

PIC values were then used to calculate a RAPD Primer Index (RPI), ISSR Primer Index (IPI) and SSR Primer Index (SPI) which were generated by multiplying the PIC values of all the markers amplified by the same primer.

3.8.2 Dendrogram Analysis

Clear and distinct bands amplified by RAPD, ISSR and SSR primers were scored for the presence (1) and absence (0) for the corresponding band among the groundnut entries. The data were entered in to MS-Excel data sheet and subsequently analyzed using NTSYS-pc version 2.02 (Rohlf, 1998).

The data matrix was read by NTSYS-pc version 2.2 (numerical taxonomy and multivariate analysis system for personal computers, exeter software) and analyzed by the SIMQUAL (similarity for qualitative data) program with Jaccard's similarity coefficient. SIMQUAL is a program for computing a variety of similarity and dissimilarity coefficients for qualitative data. The qualitative nature of the absence (0) or presence (1) state of a marker was used as the basis for similarity analysis among various maize entries. A matrix of 0 and 1 act as the input, and the output is a matrix of similarity or dissimilarity coefficients. The resultant similarity matrix was entered into

SAHN (sequential, agglomerative, hierarchical and nested clustering method) clustering program, a tree matrix was produced and a dendrogram constructed using UPGMA (unweighted pair-group method with arithmetic averages). The assumption underlying the use of UPGMA clustering is the equal rate of evolution along all dendrogram branches. Dendrogram of publication quality were produced from the output tree file of SAHN by TREE (tree display) program in graphics mode.

Clustering methods create clusters of the data, no matter whether there are true clusters in the data or not, so a check was made for the existence of true clusters. This was done by using the tree matrix produced by SAHN to calculate the cophenetic values of similarity or dissimilarity by the program COPH (cophenetic values). The cophenetic value matrix was compared with the original tree matrix for goodness of fit of the cluster analysis to the data. This type of cophenetic correlation was done by the MAXCOMP (matrix comparison) program (Rohlf, 1998). The program MAXCOMP plots the cophenetic value matrix against the original tree matrix, and computes the cophenetic correlation coefficient (r) and the Mantel test statistic (Z).

<u>The test criterion of Mantel test is as follows</u>:

$$Z = \sum_{i<j}^{n} X_{ij}Y_{ij}$$

X_{ij} = off-diagonal elements of cophenetic value matrix

Y_{ij} = off-diagonal elements of original tree matrix

n = the number of elements of the matrices

As the cophenetic correlation coefficient is positively correlated to the mantel test statistic and in standardized units, it is easier to use it as a measure of goodness of fit for a cluster analysis than the mantel test statistic. The degree of fit can be referred as follows (Rohlf, 1998):

Level	**Degree of Fit**
$0.9 \leq r$	Very good fit
$0.8 \leq r < 0.9$	Good fit
$0.7 \leq r < 0.8$	Poor fit
$r < 0.7$	Very poor fit

CHAPTER - IV

RESULTS AND DISCUSSION

The experiment was conducted on **"Genetic diversity analysis in groundnut (*Arachis hypogaea* L.) based on molecular markers and biochemical parameters".** The results obtained on biochemical analysis and molecular marker analysis in semi-spreading, spreading and bunch varieties of groundnut which are popular and grown in this region are presented and discussed under this chapter. Total 18 varieties of groundnut were taken for this study among the three groups of groundnut and 6 varieties were selected from each group.

4.1 Biochemical parameters

4.2 Molecular Markers

4.1 BIOCHEMICAL PERAMETERS

The biochemical analysis was done from the seeds of 18 groundnut varieties including spreading, semi-spreading and bunch varieties. The various biochemical parameters like moisture, oil, total carbohydrate, total protein, true protein, total soluble sugar, reducing sugar, non-reducing sugar and ash were studied from all varieties. Data on all the biochemical parameters studied among groundnut varieties are presented in Table 4.1.

4.1.1 Moisture content

The moisture content of 18 different groundnut varieties varied significantly. There was a considerable and significant variation among the groundnut varieties in moisture content. The spreading group's variety GG-41 had significantly the highest 9.12% moisture content, while bunch group's variety JL-501 which had 8.62% moisture content (Table 4.1). The moisture content of the semi-spreading group variety KDG-123 had the lowest (5.59%) moisture while KDG-128 variety which was at par with semi-spreading group variety KDG-128 (6.33%).

Similarly, Eugene and Juliana (2003) studied the effects of heat processing methods on the proximate composition, caloric value, mineral concentrations, vitamins A and C levels and lipid characterization of seed pastes of *Arachis hypogaea*. The groundnut seed contain moisture 4.58 ± 0.24%. Ayoola *et al.* (2012) also performed proximate and mineral analysis of groundnut (*Arachis hypogaea* L.) seeds on dry

weight basis. The consequence showed that the groundnut seeds contain moisture content of 7.40%.

4.1.2 Oil Content

Data on oil per cent of the 18 groundnut varieties determined found to be significant. There was a substantial difference in the group of groundnut types. The value for oil content varied significantly among the groundnut varieties and ranged from 46.16 to 53.56% (Table 4.1). The variety GJG 32 of the bunch group had the highest oil content with 53.56% followed by the semi-spreading variety KDG-123 with 52.14% oil content. The semi-spreading variety Kaushal had the lowest oil content (46.15%) which was at par with GG-11(46.85%) and GG-16 (46.77%) varieties of spreading group, GG-HPS-2 (46.86%) of the semi-spreading group and JL-501 (46.64%) of the bunch group.

Similarly, Asibuo *et al*. (2008) determined nutritional quality of 20 groundnut varieties grown in Ghana. The oil content of Virginia cultivars belonging to subspecies hypogaea was greater (49.7%) than that of Spanish and Valencia market kinds belonging to subspecies fastigiata (47.3%). Mora-Escobedo *et al*. (2015) who study on eight peanut (*Arachis hypogaea*) cultivars that were grown in Mexico and were analyzed for the physical and chemical characteristics of their seeds and for the physicochemical properties and fatty acid profiles of their oils to select the most promising candidate in terms of oil stability and nutrient composition. The results showed that the oil varied from 37.9% to 56.3%. The major fatty acids found in the oil samples were palmitic (11.9-13.2%), oleic (45.2-53.8%) and linoleic (25.1-29.2%) acids. Atasie *et al*. (2009) also did proximate, physico-chemical and elemental analysis of groundnut. The results showed that the groundnut oil contained 47.00%.

4.1.3 Total Carbohydrate

Data on total carbohydrate content varies significantly among 18 varieties of groundnut. The data on total carbohydrate content ranged between 14.49-22.96% among groundnut varieties (Table 4.1). The variety GG-HPS-2 of the semi-spreading group had the highest carbohydrate content (22.96%) which was at par with the Kaushal with 22.44%. GJG 32, a bunch group variety, had the lowest carbohydrate content (14.49%), which was at par with the KDG-123 with 15.17%.

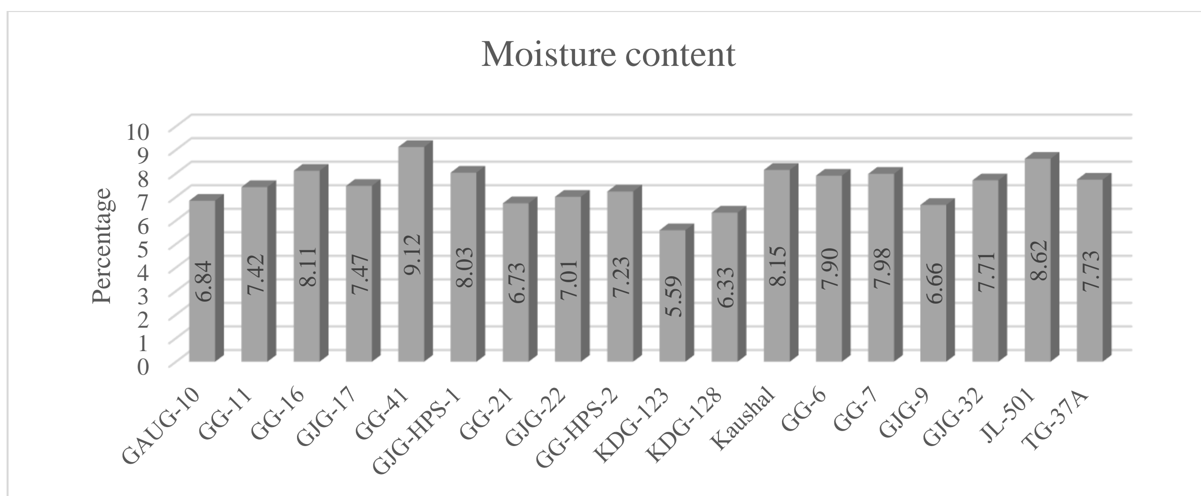


Fig. 4.1: Comparative moisture content among 18 groundnut varieties

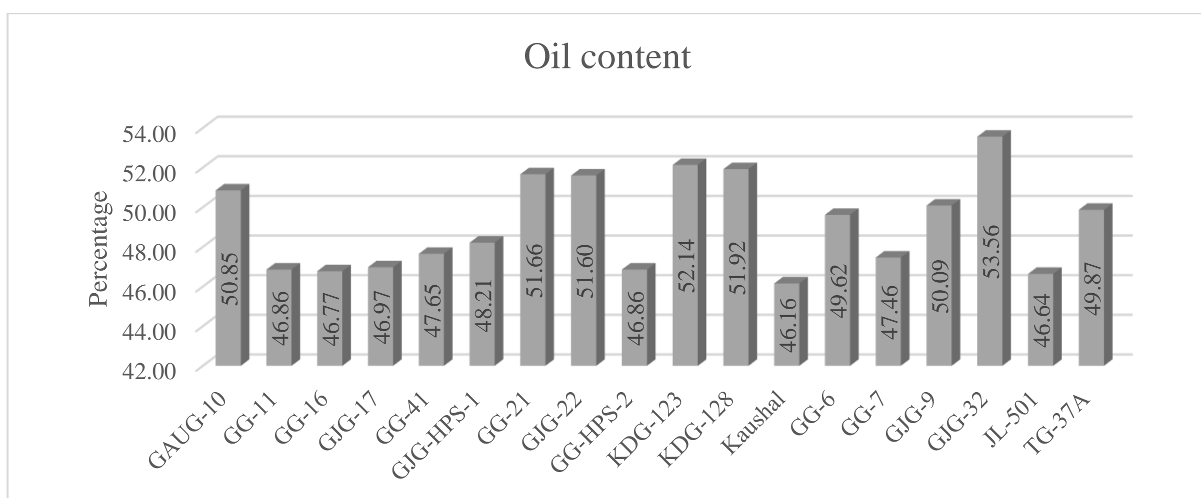


Fig. 4.2: Comparative oil content among 18 groundnut varieties

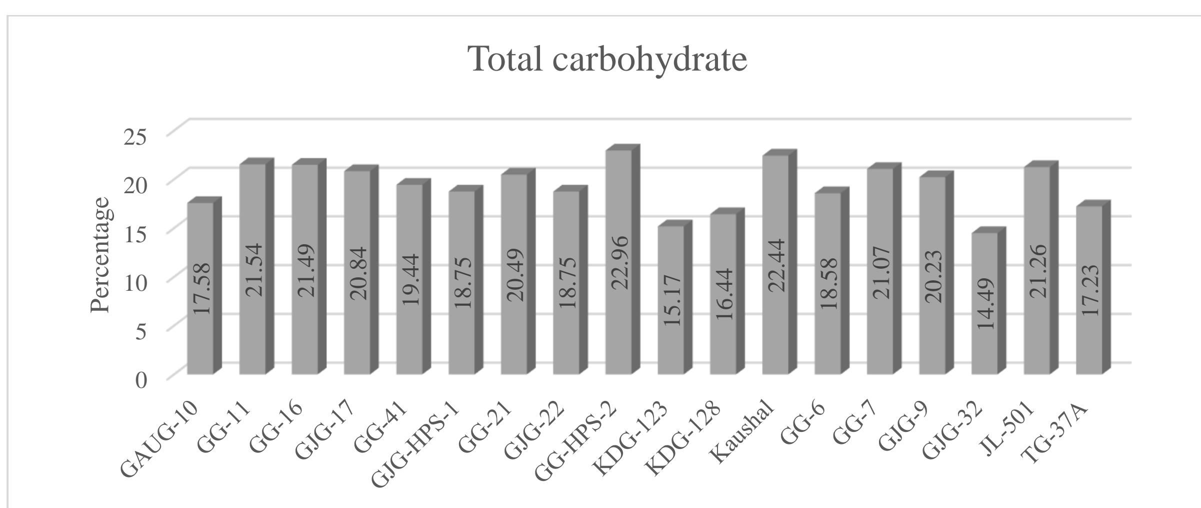


Fig. 4.3: Comparative total carbohydrate among 18 groundnut varieties

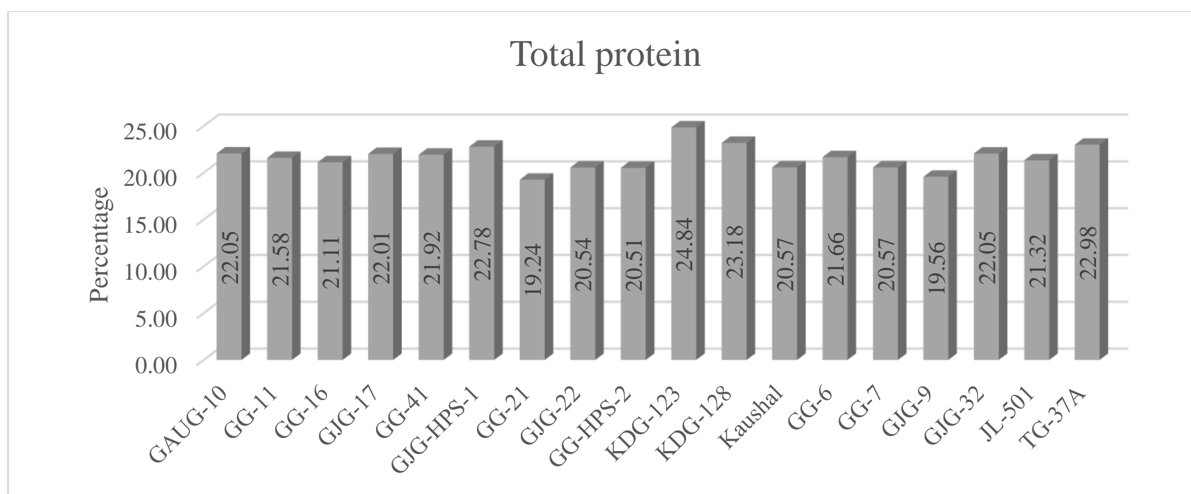


Fig. 4.4: Comparative total protein among 18 groundnut varieties

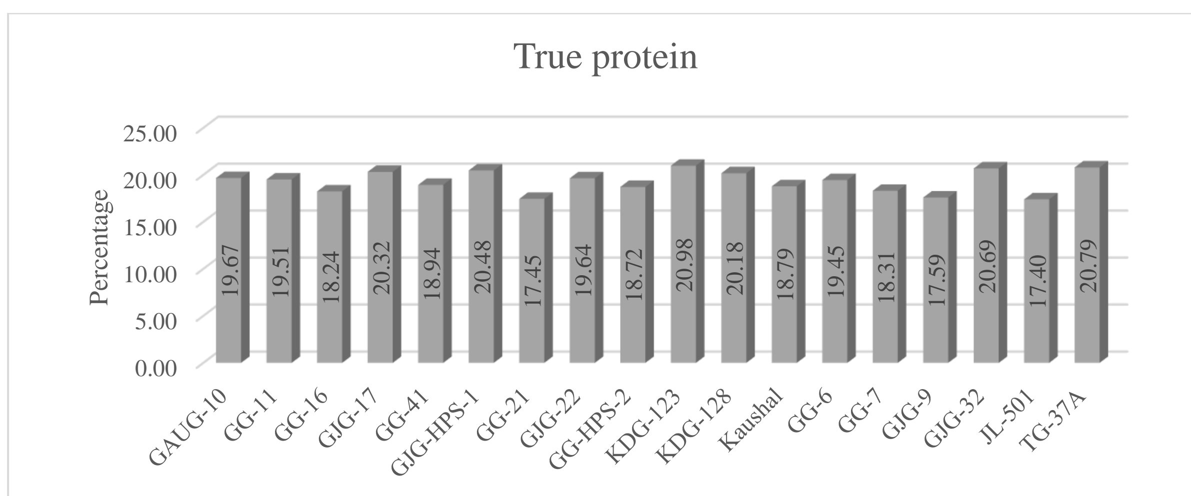


Fig. 4.5: Comparative true protein among 18 groundnut varieties

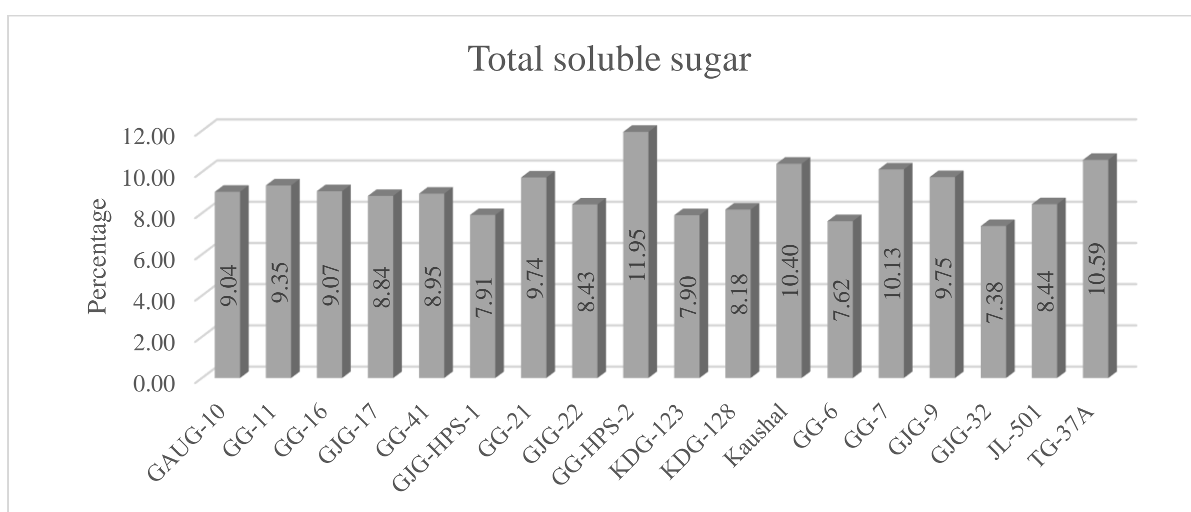


Fig. 4.6: Comparative total soluble sugar among 18 groundnut varieties

The results are in full agreement with the result of Ingale and Shrivastav (2011) who made analysis for proximate composition, anti-nutritional and nutritional value of seeds of new variety of groundnut (*Arachis hypogaea* L) JL-24 was determined. The groundnut seed included carbohydrate (21.26%). Evans *et al*. (2015) also worked on twenty-four (24) fresh groundnut samples using standard methods and procedures. The consequence indicated average carbohydrate contents of 19.16% for fresh groundnuts.

4.1.4 Total Protein

Results of total protein content of the 18 groundnut varieties found to differ significantly. The data on total protein content showed significant differences and ranged between 19.24-24.84% among the groundnut varieties (Table 4.1). The variety KDG-123 of the semi-spreading group had significantly the highest protein content value 24.84% followed by KDG-128 and TG-37A with 23.18% and 22.98% total protein, respectively. The spreading group variety GG-21 had the lowest protein content value 19.24% which was at par with GJG-9 19.56% protein content, while GG-HPS-2 was the followed with 20.51% protein content.

The results are quite relevant to the results of Campus *et al*. (2009) who studied agricultural yield, chemical composition (protein, fat, carbohydrates, fiber and ash), amino acid profile, digestibility, fatty acid profile, tocopherol and sterol contents. They observed protein level ranged from 23.5 to 26.6%. Ingale and Shrivastav (2011) who analysed proximate composition, anti-nutritional and nutritional value of seeds of new variety of groundnut (*Arachis hypogaea* L) JL-24 was determined. The groundnut seed included crude protein (25.20%). Mora-Escobedo *et al*. (2015) made study on eight peanut (*Arachis hypogaea*) cultivars that were analyzed for the physical and chemical characteristics of their seeds. The results showed that the protein ranged from 28.5% to 32.9%.

4.1.5 True Protein

Eighteen varieties of groundnut were differed in case of true protein content (%). There was a significant difference in the true protein content among groundnut varieties. True protein content of groundnut varieties ranged between 17.40-20.98% (Table 4.1). The variety KDG-123 of the semi spreading group had the maximum protein content value 20.98% which was at par with TG-37A (20.79%),

GJG-32 (20.69%) and GJG-HPS-1 (20.48%). The bunch group variety JL-501 had the minimum protein content value with 17.40% followed by GG-21 with 17.45%.

Similarly, Muhammad *et al*. (2009) determined biochemical composition and some phytochemicals in the seeds of 4 groundnut (*Arachis hypogaea* L.) varieties *viz*., Golden, Barri 2000, Mongphalla and Mongphalli 334 cultivated in arid zones of Pakistan. The contents of water soluble and salt soluble protein fractions ranged from 2.23 ± 0.23 to 2.63 ± 00.13 g/100g and 15.00 ± 0.09 to 16.38 ± 0.21 g/100g, respectively.

4.1.6 Total Soluble Sugar

The 18 varieties of groundnut were analyzed for total soluble sugar content (%). Significant difference were observed, among the varieties. The data on total soluble sugar content varied significantly and ranged between 7.38-11.95% (Table 4.1). The semi spreading group of the variety GG-HPS-2 had the highest TSS content (11.95%). The GJG-32 variety's bunch group had the lowest TSS content (7.38%) which was at par with GG-6 with 7.62% total soluble sugar content.

Sharma *et al.* (2019) analyzed seed quality traits in fifty-eight groundnut germplasms. They found a wide variation for total soluble sugars (TSS) content and it was in the range of 4.37 to 7.22%. Muhammad *et al*. (2009) determined biochemical composition and some phytochemicals in the seeds of 4 groundnut (*Arachis hypogaea* L.) varieties *viz*., Golden, Barri 2000, Mongphalla and Mongphalli 334 cultivated in arid zones of Pakistan. The contents of total sugar ranged from 7.64 ± 0.06 to 8.30 ± 0.12 g/100g. Our results are quite relevant with the results of Sharma *et al*. (2019) and Muhammad *et al*. (2009).

4.1.7 Reducing Sugar

Significant difference were found in the groundnut varieties for reducing sugar content (%). The bunch group variety TG-37A had more reducing sugar content value 3.05% which was at par with GJG-9 and Kaushal having 2.99% and 2.93% reducing sugar, respectively. The semi-spreading group of variety GJG-22 had the lowest reducing sugar content value which 1.67% followed by GJG-HPS-1 with 1.93% (Table 4.1).

The results are in complete agreement with the result of Muhammad *et al*. (2009) who determined biochemical composition and some phytochemicals in the seeds

Table 4.1: Data on biochemical parameters of 18 groundnut varieties

Sr. No.	Varieties	Moisture content (%)	Oil content (%)	Total carbohydrate (%)	Total protein (%)	True protein (%)	Total soluble sugar (%)	Reducing sugar (%)	Non-reducing sugar (%)	Ash (%)
1	GAUG-10	6.87	50.85	17.58	22.05	19.67	9.04	2.60	6.44	2.71
2	GG-11	7.42	46.85	21.54	21.58	19.51	9.35	2.27	7.08	2.17
3	GG-16	8.11	46.77	21.49	21.11	18.24	9.07	2.23	6.84	2.33
4	GJG-17	7.47	46.97	20.84	22.01	20.32	8.84	2.91	5.93	2.15
5	GG-41	9.12	47.65	19.44	21.92	18.94	8.95	1.99	6.96	1.64
6	GJG-HPS-1	8.03	48.21	18.75	22.78	20.48	7.91	1.93	5.98	1.85
7	GG-21	6.73	51.66	20.49	19.24	17.45	9.75	2.50	7.24	1.72
8	GJG-22	7.01	51.60	18.75	20.54	19.64	8.43	1.67	6.76	2.13
9	GG-HPS-2	7.23	46.86	22.96	20.51	18.72	11.95	2.39	9.56	2.32
10	KDG-123	5.59	52.14	15.17	24.84	20.98	7.90	2.51	5.40	2.31
11	KDG-128	6.33	51.92	16.44	23.18	20.18	8.18	2.62	5.57	2.18
12	Kaushal	8.15	46.16	22.44	20.57	18.79	10.40	2.93	7.47	2.25
13	GG-6	7.90	49.62	18.58	21.66	19.45	7.62	2.02	5.59	2.21
14	GG-7	7.98	47.46	21.07	20.57	18.31	10.13	2.71	7.42	2.64
15	GJG-9	6.66	50.09	20.23	19.56	17.59	9.75	2.99	6.76	3.27
16	GJG-32	7.71	53.56	14.49	22.05	20.69	7.38	2.51	4.87	2.14
17	JL-501	8.62	46.64	21.26	21.32	17.40	8.44	2.23	6.21	2.08
18	TG-37A	7.73	49.87	17.23	22.98	20.79	10.59	3.05	7.54	2.12
S.Em.±		**0.15**	**0.44**	**0.36**	**0.20**	**0.12**	**0.09**	**0.05**	**0.11**	**0.05**
C.D. at 5 %		**0.42**	**1.26**	**1.04**	**0.58**	**0.35**	**0.28**	**0.14**	**0.32**	**0.15**
C.V. %		**3.39**	**1.55**	**3.26**	**1.65**	**1.11**	**1.87**	**3.63**	**2.96**	**4.06**

of 4 groundnut (*Arachis hypogaea* L.) varieties *viz*., Golden, Barri 2000, Mongphalla and Mongphalli 334 cultivated in arid zones of Pakistan. The contents of reducing sugar between 1.64 ± 0.11 to 1.97 ± 0.08 g/100g.

4.1.8 Non-Reducing Sugar

The non-reducing sugar content (%) of 18 varieties of groundnut were differed significantly. Non reducing sugar varied significantly between 4.87 to 9.56% among the groundnut varieties (Table 4.1). The semi-spreading group of the variety GG-HPS-2 had significantly the maximum non-reducing sugar content value, with 9.56% which was followed and par with bunch group variety TG-37A with 7.54% non-reducing sugar content. The minimum non-reducing sugar content value 4.87% was found in the GJG-32 bunch group variety which was followed by semi-spreading variety KDG-123 with 5.40% non-reducing sugar content.

The result are akined with by Muhammad *et al*. (2009) who determined biochemical composition and some phytochemicals in the seeds of 4 groundnut (*Arachis hypogaea* L.) varieties *viz*., Golden, Barri 2000, Mongphalla and Mongphalli 334 cultivated in arid zones of Pakistan. Non-reducing sugar ranged from 5.67 ± 0.21 to 6.67 ± 0.24 g/100g.

4.1.9 Ash

The ash content (%) of 18 varieties of groundnut were found significant. The significant difference was observed among the group of groundnut varieties. Ash content ranged between 1.64-3.27% among the groundnut varieties. The variety GJG-9 of the bunch group had the highest ash content value 3.27% followed by GAUG-10 with 2.71%. The spreading group of variety GG 41 had the lowest ash content value with 1.64% which was at par with GG-21 with 1.72% ash content.

Similarly, Atasie *et al*. (2009) did proximate, physico-chemical and elemental analysis of groundnut. Groundnut contained 3.08% ash. Ingale and Shrivastav (2011) also analysed proximate composition, anti-nutritional and nutritional value of seeds of new variety of groundnut (*Arachis hypogaea* L) JL-24 was determined. Ayoola *et al*. (2012) also performed proximate analysis of groundnut (*Arachis hypogaea* L.) seeds. The consequence showed that the groundnut seeds ash content of 1.48%. The groundnut seed included ash value 2.57%. So, one results are matured with the results of these researchers.

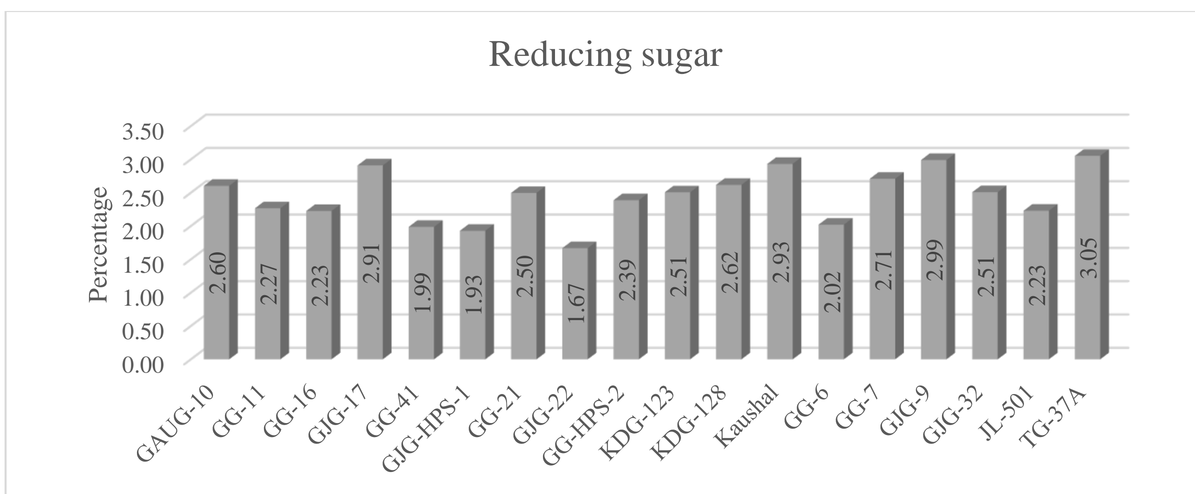


Fig. 4.7: Comparative reducing sugar among 18 groundnut varieties

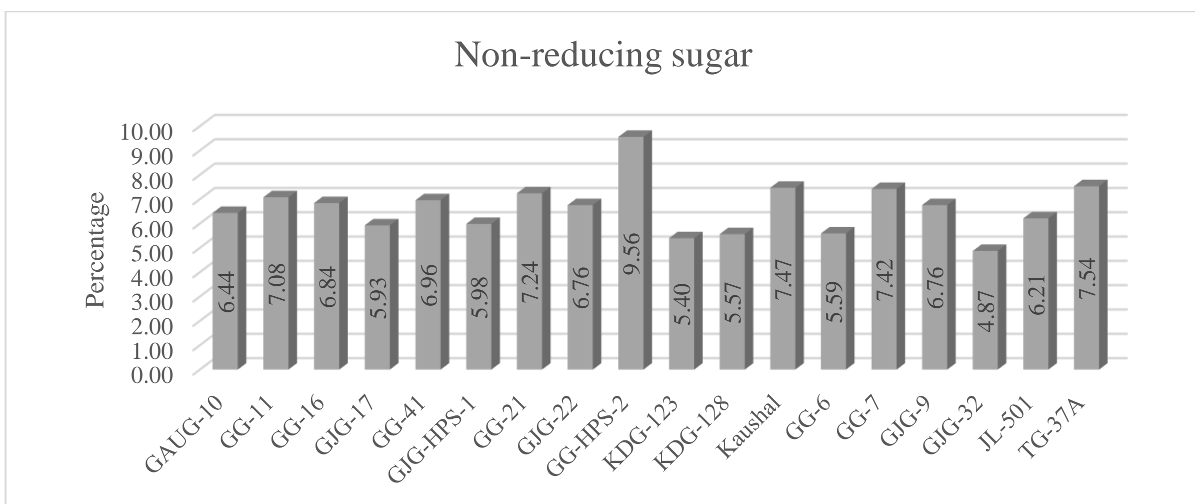


Fig. 4.8: Comparative non-reducing sugar among 18 groundnut varieties

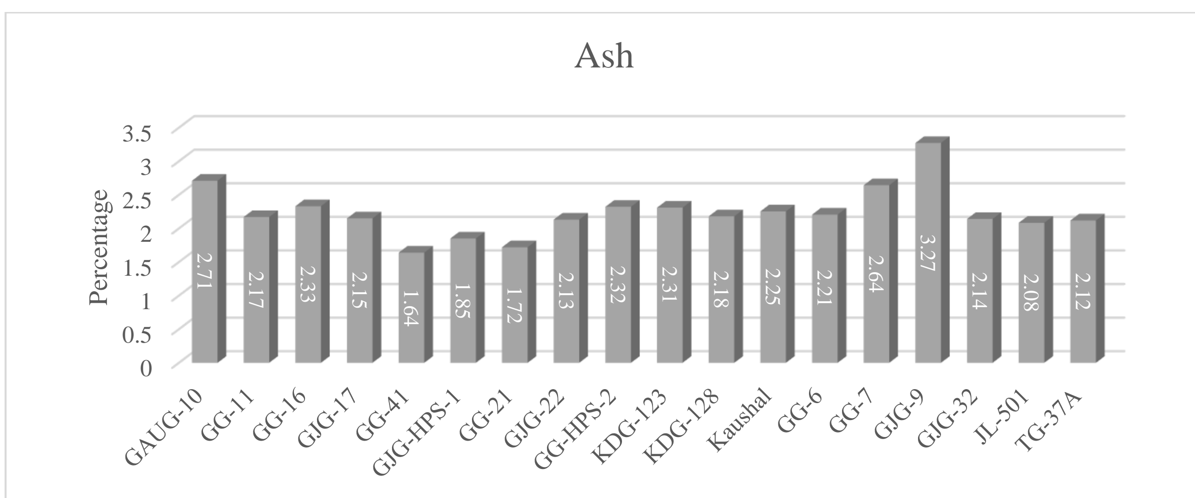


Fig. 4.9: Comparative ash content among 18 groundnut varieties

4.2 MOLECULAR CHARACTERIZATION

Total 18 groundnut varieties including spreading, semi-spreading and bunch type were analyzed for the molecular characterization using RAPD, ISSR and SSR molecular markers with an aim to find out phylogenic relationship among them.

4.2.1 Random Amplified Polymorphic DNA (RAPD) Analysis

The RAPD analysis was used for characterization of 18 different groundnut varieties for studying genetic diversity and similarity among different varieties. In present investigation, 18 varieties were subjected to RAPD analysis using 21 different decamer primers of operon series such as OPA, OPC, OPD, OPN, OPZ, OPQ, OPL and CMN.

4.2.1.1 Polymorphism pattern of RAPD

Total 46 RAPD primers were screened, out of which 21 primers amplified a total of 118 bands. The RAPD markers OPC-05 produced maximum number of 10 bands, while CMN-A44 produced minimum number of 1 band. Out of 118 bands, 98 bands were polymorphic with an average of 4.7 bands per primer, while 20 bands were monomorphic. Among the 98 polymorphic bands, 91 bands were shared polymorphic, while 7 bands were unique-polymorphic (Table 4.2).

The per cent polymorphism obtained for RAPD primers were ranged from 0.00 to 100.00% with an average value of 81.03% per primer. The amplified fragments were in the range of 136-2680 bp. The smallest fragment of 136 bp was amplified by OPQ-14 demonstrated a shortest distance between two adjacent microsatellites and the largest fragment of 2680 bp was amplified by OPD-05 represented the longest distance between two microsatellites among all the studied varieties. OPN-10 primer produced highest 11 bands (allele), while CMN-A44 produced lowest 1 band. The polymorphism information content (PIC) values for RAPD markers were ranged from 0.00 to 0.95 with an average value of 0.78 per primer and RAPD primer index (RPI) differed from 0 to 9.68 with an average value of 4.64 as presented in Table 4.2. The performance of individual primer to amplify genomic DNA of 18 groundnut varieties are discussed as under.

Table 4.2: Size, number of amplified bands, per cent polymorphism and PIC obtained by RAPD primers in 18 groundnut varieties

Sr. No.	RAPD Primer	Band Size (bp)	Total No. of Bands (A)	Polymorphic Bands (B)			Mono-morphic Band	% Poly-morphism (B/A)	PIC	RPI
				S	U	T				
1	OPA-16	168-1325	5	2	2	4	1	80.00	0.75	3.75
2	OPC-05	137-1616	10	8	0	8	2	80.00	0.89	8.90
3	OPD-05	357-2680	5	3	0	3	2	60.00	0.76	3.80
4	OPL-01	171-2424	8	5	1	6	2	75.00	0.84	6.72
5	OPL-04	182-634	4	4	0	4	0	100.00	0.75	3.00
6	OPN-04	297-999	6	5	0	5	1	83.33	0.83	4.98
7	OPN-10	189-1480	11	9	2	11	0	100.00	0.88	9.68
8	OPN-13	167-1437	7	4	0	4	3	57.14	0.86	6.02
9	OPN-16	274-1778	5	4	0	4	1	80.00	0.74	3.70
10	OPQ-14	136-875	8	8	0	8	0	100.00	0.87	6.96
11	OPZ-10	548-1214	6	6	0	6	0	100.00	0.83	4.98
12	OPZ-11	240-1872	4	4	0	4	0	100.00	0.88	3.52
13	OPZ-19	250-1987	9	5	2	7	2	77.78	0.86	7.74
14	CMN-A01	266-1386	5	5	0	5	0	100.00	0.95	4.75
15	CMN-A11	183-1414	4	4	0	4	0	100.00	0.75	3.00
16	CMN-A24	311-952	4	3	0	3	1	75.00	0.77	3.08
17	CMN-A26	475-1568	3	3	0	3	0	100.00	0.75	2.25
18	CMN-A37	809-1186	3	1	0	1	2	33.34	0.82	2.46
19	CMN-A40	273-878	3	0	0	0	3	0.00	0.67	2.01
20	CMN-A44	615	1	1	0	1	0	100.00	0.00	0.00
21	CMN-A55	160-1449	7	7	0	7	0	100.00	0.87	6.09
TOTAL			118	91	7	98	20	-	-	-
AVERAGE			-	-	-	4.7	0.95	81.03	0.78	4.64

S = Shared**; U** = Unique**; T** = Total polymorphic bands; **PIC** = Polymorphism information content; **IPI** = ISSR primer index = Number of bands x PIC

OPA-16

The primer OPA-16 amplified total 5 amplicons with size ranging from 168-1325 bp. Among which 2 fragments was shared polymorphic, 2 unique band and 1 fragment was monomorphic having 80.00% polymorphism and PIC value 0.75 (Table 4.2).

OPC-05

Total 10 number of amplified fragments were produced by primer OPC-05 with size ranged between 137-1616 bp of which 8 fragments were shared polymorphic with no unique band and 2 fragment were monomorphic having 80.00% polymorphism and PIC value 0.89 (Table 4.2).

OPD-05

Total 5 number of amplicons were amplified by the primer OPD-05 with size ranging from 357-2680 bp. The fragment which had highest 2680 bp band size which was the maximum among all the fragments amplified by all 21 primers. Three fragment was shared polymorphic and 2 fragments were monomorphic having 60.00% polymorphism and PIC value 0.76. This primer did not produce any unique band (Table 4.2).

OPL-01

The OPL-01 primer amplified total 8 fragments with size ranging from 171-2424 bp of which 5 fragments were shared polymorphic, 1 fragment was unique and 2 fragment were monomorphic having 75.00% polymorphism with PIC value 0.84 (Table 4.2).

OPL-04

Four fragments were obtained by amplification of primer OPL-04 with size ranging from 182-634 bp of which all 4 fragments were shared polymorphic, this primer did not give any unique or monomorphic band and having 100.00% polymorphism with PIC value 0.75 (Table 4.2).

OPN-04

The OPN-04 primer amplified total 6 fragments with size ranging from 297-999 bp of which 5 fragments were shared polymorphic and 1 fragment was monomorphic having 83.33% polymorphism with PIC value 0.83. This primer did not have any unique band (Table 4.2).

OPN-10

Total 11 which was maximum fragments among all primers tested were obtained by amplification of primer OPN-10 with size ranging from 189-1480 bp out of which 9 fragments were shared polymorphic and 2 unique bands having 100.00% polymorphism and PIC value 0.88 (Table 4.2). This primer did not show any monomorphic band.

OPN-13

Seven fragments were amplified by the primer OPN-13 with size ranging from 167-1437 bp among which 4 fragment was shared polymorphic and 3 fragments were monomorphic having 57.14% polymorphism and PIC value 0.86. This primer did not have any unique band (Table 4.2).

OPN-16

The primer OPN-16 amplified total 5 amplicons with size ranging from 274-1778 bp. Out of which 4 fragments was shared polymorphic and 1 fragment was monomorphic having 80.00% polymorphism and PIC value 0.74 (Table 4.2). This primer did not have any unique band.

OPQ-14

Total 8 fragments were amplified by primer OPQ-14 with small size ranging from 136-875 bp and all 8 fragments were shared polymorphic, it did not show any unique or monomorphic band and having 100.00% polymorphism with PIC value 0.87 (Table 4.2).

OPZ-10

Six fragments were amplified by primer OPZ-10 having band size ranging from 548-1214 bp. All the 6 fragments were shared polymorphic bands and no monomorphic band, having 100.00% polymorphism and PIC value 0.83 (Table 4.2). This primer did not produce any unique band.

OPZ-11

Total 4 fragments were amplified by primer OPZ-11 having band size ranging from 240-1872 bp. All the 4 fragments were polymorphic, and all were shared polymorphic bands and no monomorphic band, having 100.00% polymorphism and PIC value 0.88 (Table 4.2). This primer did not show any unique band.

OPZ-19

Nine fragments were amplified by the primer OPZ-19 with size ranging from 250-1987 bp among which 5 fragments were shared polymorphic, 2 fragments were

unique and 2 fragments were monomorphic having 77.78% polymorphism and PIC value 0.86 (Table 4.2).

CMN-A01

Five fragments were obtained by amplification of primer CMN-A01 with size ranging from 266-1386 bp of which all 5 fragments were shared polymorphic, this primer did not give any unique or monomorphic band and having 100.00% polymorphism with PIC value 0.95 (Table 4.2).

CMN-A11

The CMN-A11 primer amplified 4 fragments with size varied from 183-1414 bp out of which 4 fragments were polymorphic with all shared polymorphic fragments having 100.00% polymorphism and PIC value 0.75 (Table 4.2). This primer did not show any unique or monomorphic band.

CMN-A24

Four fragments were amplified by the primer CMN-A24 with size ranging from 311-952 bp among which 3 fragments were shared polymorphic and 1 fragment was monomorphic having 75.00% polymorphism and PIC value 0.77. This primer did not have any unique band (Table 4.2).

CMN-A26

The primer CMN-A26 primer produced total 3 amplicons with size ranging from 475-1568 bp and all the 3 fragments were shared polymorphic having 100.00% polymorphism and PIC value 0.75. This primer did not amplify any unique or monomorphic band (Table 4.2).

CMN-A37

Three fragments were amplified by the primer CMN-A37 with size ranging from 809-1186 bp among which 1 fragment was shared polymorphic and 2 fragments were monomorphic having 33.34% polymorphism and PIC value 0.82. This primer did not have any unique band (Table 4.2).

CMN-A40

The CMN-A40 primer gave total 3 fragments with size ranging from 273-878 bp and all fragments were monomorphic having 0.00% polymorphism and PIC value 0.67. This primer did not produce any unique or shared polymorphic band (Table 4.2).

CMN-A44

The lowest number of 1 amplicon was generated by primer CMN-A44 with fragment size 615 bp. One fragment was shared polymorphic, this primer did not show

any unique band or monomorphic band, observed 100.00% polymorphism and PIC value 0 (Table 4.7).

CMN-A55

The CMN-A55 primer produced total 7 amplicons with size ranged between 160-1449 bp and all the fragments were shared polymorphic having 100.00% polymorphism and PIC value 0.87. This primer did not show any unique or monomorphic band (Table 4.2).

Similarly, Al-Saghir and Abdel-Salam (2015) studied twenty peanut accessions that were analysed using RAPD molecular markers. Twenty-seven RAPD primers resulted in 210 amplification products, with 80 (36.4%) of them being polymorphic. In conclusion, our work established the use of RAPD markers in determining the level of genetic diversity in peanut germplasm by successfully fingerprinting peanut accessions using these markers. Patel *et al*. (2015) also investigated RAPD showing reproducible and polymorphic patterns were chosen for cultivar identification and generated a total of 103 bands/alleles in which 96 bands were polymorphic that was equal to 93.32% polymorphism and 7 bands were monomorphic. The average bands per primer were 4.68. The PIC values varied between 0.7 (OPB-09) and 0.878 (OPC-13) with an average of 0.661 per primer. Among the screened primers OPA-05, OPA-10, OPA-15 and OPB-11 showed variety specific markers, while OPB-09, OPB-11 OPC-11, OPL-12 and OPO-11 produced species specific DNA fragments.

The present results are in full agreement with the results of Suneetha *et al*. (2015) examined genetic diversity among 12 varieties of groundnut using RAPD markers. Seven polymorphic RAPD markers were discovered among the 60 RAPD markers analyzed. In all, 58 amplification products were discovered, with 48 alleles being polymorphic (82.9%). OPJ 6 was the most polymorphic of the markers, with 11 alleles, nine of which were polymorphic. The OPJ 4 marker amplified the fewest number of alleles.

4.2.1.2 Identification of genotype specific marker from RAPD primers

Among the screened primers, the primers OPA-16, OPL-01, OPN-10 and OPZ-19 each amplified a unique band which was variety specific band (Table 4.3). Primer OPL-01 produced one unique band which was 986 bp for the variety KDG-123. Primer OPA-16 produced two unique bands which was 1325 bp and 908 bp for the variety

GJG-22, primer OPN-10 produced two unique bands which had 917 bp and 254 bp band size for the variety GJG-HPS-1 and primer OPZ-19 produced two unique bands having band size 1439 bp and 477 bp for the variety GJG-32 and Kaushal, respectively.

4.2.1.3 Genetic similarity

Genetic similarity was determined for each pair of eighteen varieties by Jaccard's similarity coefficient which revealed that the lowest similarity of 54% was noticed betweenGG-HPS-2 and JL-501, while the highest similarity of 91% was noticed between GJG-17 and GG-41 varieties (Table 4.4).

4.2.1.4 Cluster Analysis of RAPD

The dendrogram was constructed using UPGMA based on Jaccard's similarity coefficient through NTSYSpc-2.02i software for RAPD data of 18 groundnut varieties (Table 4.4 and Fig. 4.10).

The 18 groundnut entries were grouped into two main clusters: cluster-I and cluster-II, which shared 64% similarity. The cluster-I was divided into two subclusters-A and B both contained a total of 17 varieties (Fig. 4.10). Subcluster-A was further bifurcated into two groups A1 and A2 which had nearly 72% likeness. Group A2 consisted of two varieties such as GG-16 and GG-21 having nearly 82% similarity. Group A1 was further divided in two group A1(a) and A1(b). Group A1(a) consisted of twelve entries such as GAUG-10, GJG-17, GG-41, GJG-32, GJG-22, KDG-128, Kaushal, GG-6, GG-7, GJG-9, GJG-HPS-2 and KDG-123 having nearly 78% similarity, while group A1(b) contained of only one variety GG-11.

The cluster-II consisted of only one variety GG-HPS-2 that was the most diversified variety among all eighteen varieties.

To test the goodness of fit of the clustering of RAPD data, matrix of cophenetic values were also computed using the program COPH. The cophenetic matrixes were compared to the original matrixes produced by SIMQUAL. The plots of one matrix against the other and the association statistics were made and calculated by MAXCOMP. The plot and statistics of 18 groundnut varieties included in present study is shown in (Fig. 4.11). In the present investigation the mental test statistics Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation r = 0.85) as categorized by Rohlf (1998) was found under the category of **"good fit"**.

Table 4.3: Molecular weight (bp) of groundnut varieties specific markers generated by RAPD primers

RAPD Primers	Varieties																	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
OPA-16	-	-	-	-	-	-	-	1325	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	908	-	-	-	-	-	-	-	-	-	-
OPC-05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPD-05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPL-01	-	-	-	-	-	-	-	-	-	986	-	-	-	-	-	-	-	-
OPL-04	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPN-4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0PN-10	-	-	-	-	-	917	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	254	-	-	-	-	-	-	-	-	-	-	-	-
OPN-13	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPN-16	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPQ-14	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPZ-10	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPZ-11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
OPZ-19	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1439	-	-
	-	-	-	-	-	-	-	-	-	-	-	477	-	-	-	-	-	-
CMN-A01	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMN-A11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMN-A24	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMN-A26	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMN-A37	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMN-A40	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMN-A44	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
CMN-A55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	-	-	-	-	-	2	-	2	-	1	-	1	-	-	-	1	-	-

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

Table 4.4: Jaccard's Similarity Coefficient of groundnut varieties Based on RAPD data analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	0.84	1.00																
3	0.75	0.69	1.00															
4	0.88	0.82	0.76	1.00														
5	0.87	0.79	0.73	**0.91**	1.00													
6	0.77	0.68	0.72	0.81	0.82	1.00												
7	0.85	0.78	0.82	0.83	0.80	0.72	1.00											
8	0.81	0.71	0.67	0.86	0.82	0.79	0.74	1.00										
9	0.70	0.58	0.57	0.69	0.68	0.59	0.59	0.67	1.00									
10	0.76	0.71	0.60	0.81	0.79	0.75	0.69	0.79	0.61	1.00								
11	0.86	0.74	0.66	0.85	0.83	0.79	0.75	0.82	0.73	0.79	1.00							
12	0.81	0.71	0.66	0.86	0.83	0.79	0.72	0.84	0.68	0.81	0.89	1.00						
13	0.79	0.70	0.67	0.84	0.80	0.84	0.74	0.84	0.63	0.81	0.85	0.88	1.00					
14	0.80	0.74	0.68	0.87	0.83	0.77	0.75	0.82	0.65	0.77	0.88	0.85	0.89	1.00				
15	0.82	0.70	0.67	0.82	0.74	0.72	0.75	0.76	0.68	0.74	0.85	0.78	0.80	0.85	1.00			
16	0.83	0.75	0.67	0.85	0.82	0.74	0.78	0.81	0.64	0.75	0.80	0.79	0.79	0.82	0.79	1.00		
17	0.72	0.75	0.60	0.71	0.68	0.66	0.66	0.67	**0.54**	0.62	0.65	0.66	0.66	0.65	0.64	0.67	1.00	
18	0.80	0.69	0.68	0.76	0.73	0.71	0.74	0.74	0.65	0.66	0.70	0.73	0.71	0.71	0.68	0.78	0.77	1.00

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

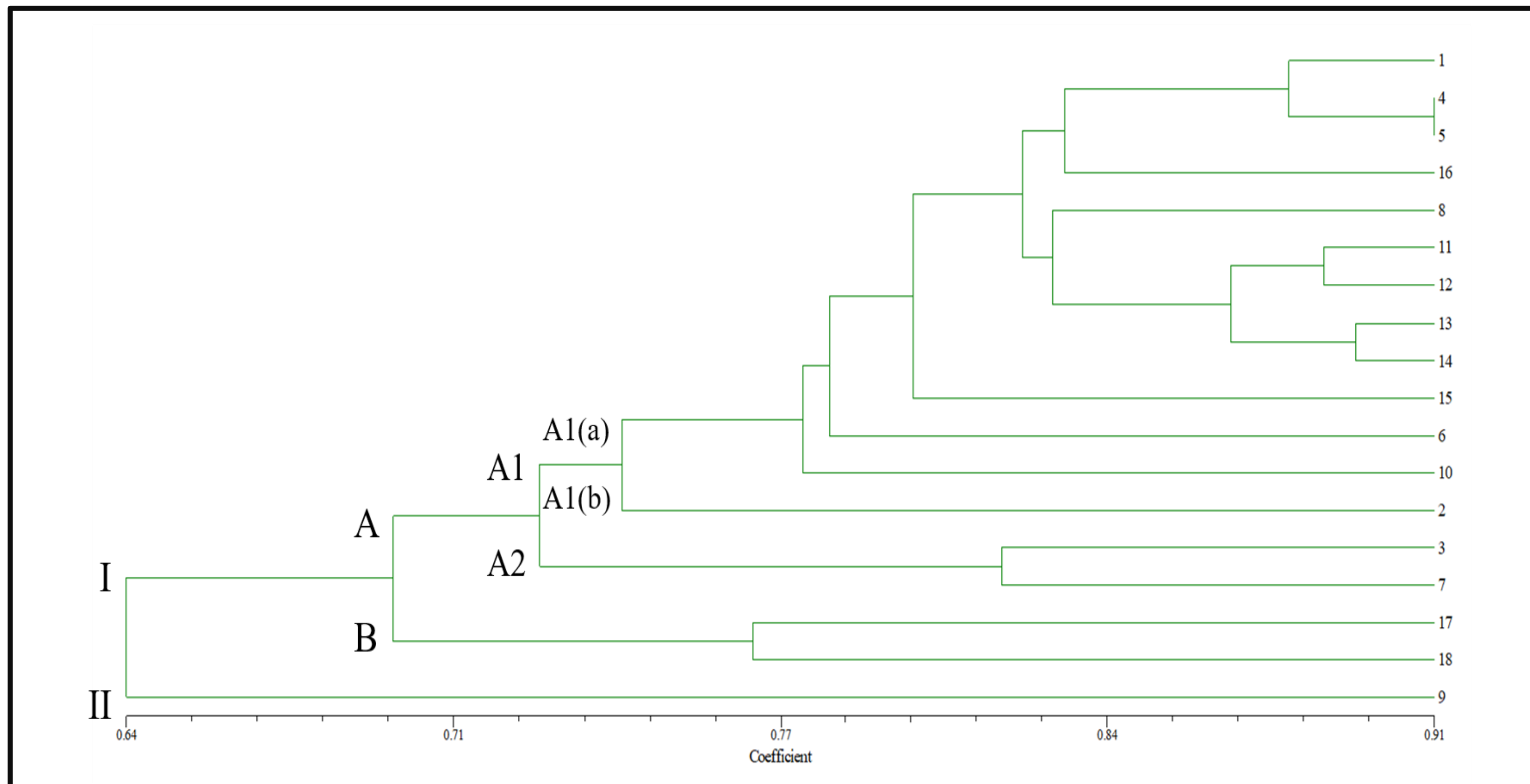
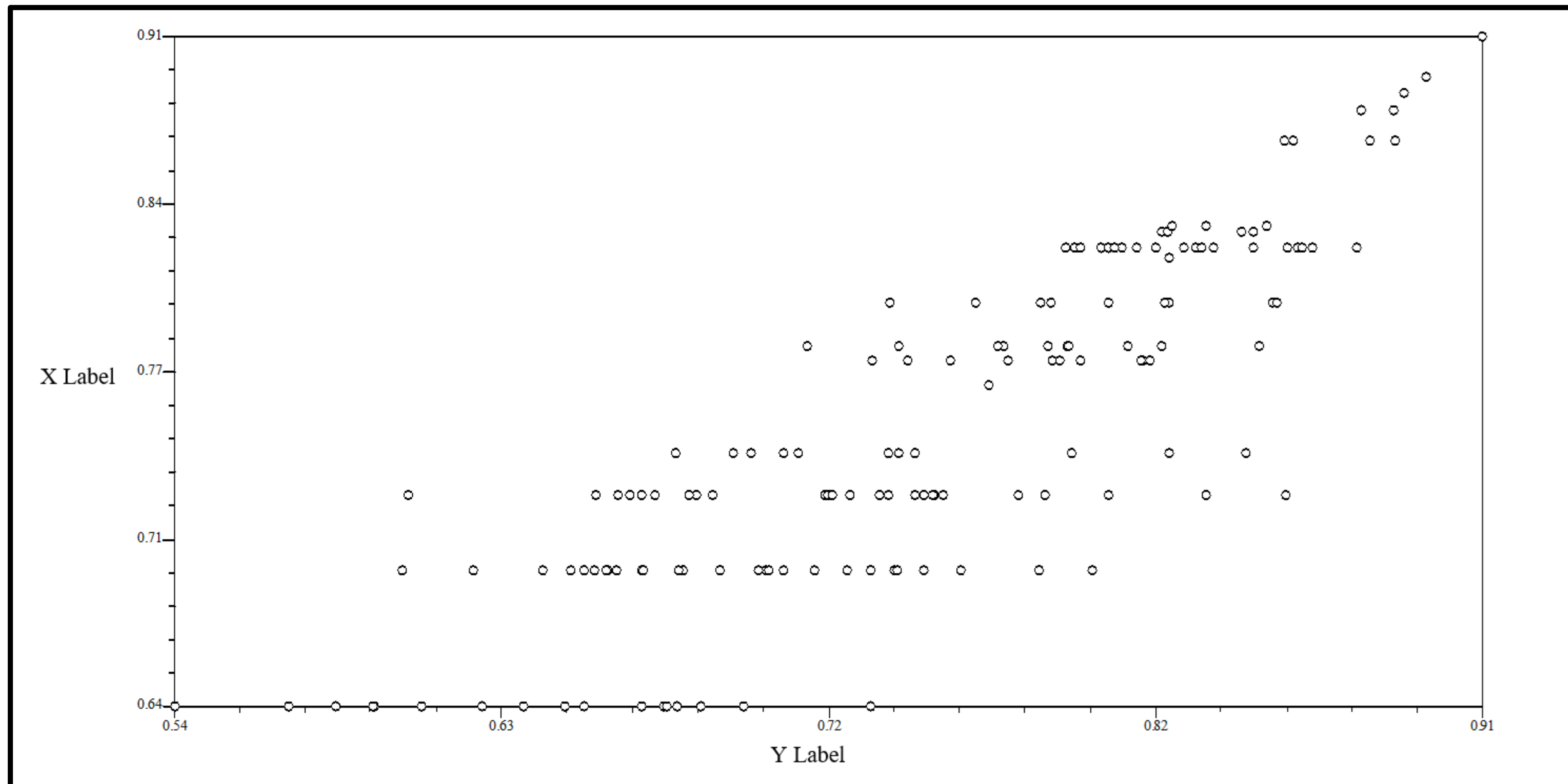


Fig. 4.10: Dendrogram depicting the genetic relationship among 18 groundnut varieties based on data of RAPD

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)



Matrix correlation: r = 0.85 (=Normalized Mantel statistic Z)

Fig. 4.11: Cophenetic values against Jaccard's similarity coefficients from RAPD data of 18 groundnut varieties

Similarly, Patel *et al*. (2015) also investigated RAPD showing PIC values varied between 0.279 (OPB-09) and 0.878 (OPC-13) with an average of 0.661 per primer. The dendrogram generated by the UPGMA method produced two main clusters, each of which contained all of the varieties grouped together in their respective sub-cluster. Kushwah *et al*. (2016) worked on 14 varieties, a total of 27 bands ranging in size from 200 bp to 2250 bp were detected, and all 27 (100%) were found to be polymorphic. Jaccard's pairwise similarity coefficients were used to determine the genetic relationship between groundnut varieties. The pairwise similarity ranges from 0.00 to 0.833. A dendrogram was plotted for the data set after data analysis using Jaccard's similarity coefficient. It showed that at genetic distance 0.00, two separate clusters with a single root were produced. The dendrogram revealed two large clusters (I-II) that were determined to be separate from each other, with just 0% similarity. Cluster I had the most genotypes (13), whereas Cluster II had only one, our results also quite relevant to this result.

The results of present investigation are also similar with the results of Kusuma *et al*. (2016) who estimated the genetic diversity and evaluated the relationship among eight groundnut varieties using Random Amplified Polymorphic DNA (RAPD). The primers used yielded 52 amplification products, 34 of which were polymorphic (65.38%). The range of Similarity matrix obtained from RAPD data of 8 groundnut varieties was 0.409-0.868. UPGMA cluster analysis found that the genotypes Narayani and Dharani were genetically related (86.8% similar), whereas Kadiri-6 and Dharani were dissimilar.

4.2.2 Inter Simple Sequence Repeat (ISSR) Analysis:

In present investigation, 18 groundnut varieties were subjected to ISSR analysis using 13 different primers of ISSR.

4.2.2.1 Polymorphism pattern of ISSR

The eighteen groundnut varieties were subjected to ISSR with 35 primers. Out of 35 primers, 13 primers amplified a total of 51 bands among which 43 bands were polymorphic with an average of 3.30 bands per primer, while 8 bands were monomorphic. Out of 43 polymorphic bands, 40 were shared polymorphic and 3 bands were unique (Table 4.5).

The per cent polymorphism obtained for ISSR primers were ranged between 0 to 100% with an average value of 72.12% per primer. The amplified fragments were in the range of 128-1629 bp. The smallest fragment of 128 bp was amplified by UBC-805 demonstrated a shortest distance between two adjacent microsatellites and the largest fragment of 1629 bp was amplified by UBC-806 represented the longest distance between two microsatellites among all the studied varieties. UBC-806 primer produced highest 8 bands (allele), while UBC-848 produced lowest 1 band.

The polymorphic information content (PIC) was calculated for each primer. The highest PIC value was 0.87 for UBC-806, while the lowest PIC value was 0 for the primer UBC-848 with an average of 0.63 per primer. ISSR primer index (IPI) differed from 0 to 6.96 with an average of 2.79 per primer (Table 4.5). The UBC-848 primer depicted the lowest IPI value (0), while UBC-806 depicted the highest IPI value (6.96).

The performance of individual primer to amplify genomic DNA of 18 groundnut varieties is discussed as under.

UBC-801

The UBC-801 primer amplified 5 fragments with size ranging from 169-688 bp and all fragments were shared polymorphic fragment having 100.00% polymorphism and PIC value 0.79. This primer did not produce any unique or monomorphic fragment (Table 4.5).

UBC-802

Total 4 fragments with size ranging from 259-1292 bp were amplified by the primer UBC-802. Among 4 fragments, 2 were shared polymorphic fragments and 2 fragments were unique having 100.00% polymorphism and PIC value 0.55. This primer did not give any monomorphic band (Table 4.5).

UBC-803

The UBC-803 primer amplified total 6 fragments with size ranged between 172-895 bp among which all fragments were shared polymorphic having 100.00% polymorphism and PIC value 0.83. This primer did not produce any unique or monomorphic band on amplification (Table 4.5).

UBC-805

The UBC-805 primer amplified 4 fragments with size ranging from 128-1028 bp out of which all fragments were shared polymorphic having 100.00% polymorphism

and PIC value 0.55 (Table 4.5). This primer did not amplify any unique or monomorphic band.

Table 4.5: Size, number of amplified bands, per cent polymorphism and PIC obtained by ISSR primers in the 18 varieties

Sr. No.	ISSR Primer	Band Size (bp)	Total No. of Bands (A)	Polymorphic Bands (B)			Mono-morphic Band	% Poly-morphism (B/A)	PIC	IPI
				S	U	T				
1	UBC-801	169-688	5	5	0	5	0	100.00	0.79	3.95
2	UBC-802	259-1292	4	2	2	4	0	100.00	0.55	2.20
3	UBC-803	172-895	6	6	0	6	0	100.00	0.83	4.98
4	UBC-805	128-1028	4	4	0	4	0	100.00	0.55	2.20
5	UBC-806	209-1629	8	6	1	7	1	87.50	0.87	6.96
6	UBC-810	420-972	2	0	0	0	2	0.00	0.50	1.00
7	UBC-811	709-1531	3	2	0	2	1	66.67	0.72	2.16
8	UBC-812	253-1247	4	4	0	4	0	100.00	0.73	2.92
9	UBC-813	675-1146	2	1	0	1	1	50.00	0.49	0.98
10	UBC-848	800	1	0	0	0	1	0.00	0.00	0.00
11	UBC-852	208-839	5	5	0	5	0	100.00	0.79	3.95
12	UBC-857	213-1270	4	4	0	4	0	100.00	0.75	3.00
13	UBC-862	378-818	3	1	0	1	2	33.34	0.65	1.95
TOTAL			51	40	3	43	8	-	-	-
AVERAGE			-	-	-	3.30	0.61	72.12	0.63	2.79

S = Shared**; U** = Unique**; T** = Total polymorphic bands; **PIC** = Polymorphism information content; **IPI** = ISSR primer index = Number of bands x PIC

UBC-806

Eight fragments were amplified by the primer UBC-806 with size ranging from 209-1629 bp among which 6 fragments were shared polymorphic, 1 fragment was unique and 1 fragment was monomorphic having 87.50% polymorphism and PIC value 0.87 (Table 4.5).

UBC-810

The UBC-810 primer gave total 2 fragments with size ranging from 420-972 bp and all fragments were monomorphic having 0.00% polymorphism and PIC value 0.50. This primer did not produce any unique or shared polymorphic band (Table 4.5).

UBC-811

Total 3 fragments were amplified by the primer UBC-811 with size ranging from 709-1531 bp from which 2 fragments were shared polymorphic and 1 fragment was monomorphic having 66.67% polymorphism and PIC value 0.72. This primer did not show any unique band (Table 4.5).

UBC-812

The primer UBC-812 produced 4 fragments with size ranged between 253-1247 bp and all the fragments were shared polymorphic having 100.00% polymorphism and PIC value 0.73. This primer did not produce any unique or monomorphic band on amplification (Table 4.5).

UBC-813

The primer UBC-813 amplified 2 fragments with size ranged between 675-1146 bp from which 1 band was shared polymorphic and 1 band was monomorphic having 50.00% polymorphism and PIC value 0.49. This primer did not produce any unique band (Table 4.5).

UBC-848

The UBC-848 primer amplified minimum number of only 1 fragment with size 800 bp of which was shared monomorphic fragments, having no unique or polymorphic fragment having 0.00% polymorphism and PIC value 0 (Table 4.5).

UBC-852

Total 5 fragments with size ranging from 208-839bp were obtained by primer UBC-852 and all fragments were shared polymorphic having 100.00% polymorphism and PIC value 0.79 (Table 4.5). This primer did not give any unique or monomorphic band.

UBC-857

Four fragments were amplified by the primer UBC-857 with size ranging from 213-1270 bp. All the fragments were monomorphic having 100.00% polymorphism and PIC value 0.75. This primer did not produce any unique or shared fragment on amplification (Table 4.5).

UBC-862

Total 3 fragments were amplified by the primer UBC-862 with size ranging from 378-818 bp from which 1 fragment was shared polymorphic and 2 fragments were monomorphic having 33.34% polymorphism and PIC value 0.65. This primer did not show any unique band (Table 4.5).

Similarly, Mohammed *et al*. (2022) worked on genetic variability and diversity of 43 accessions of *A. hypogaea* collected from different regions of Ethiopia using ISSR markers. Out of 56 reproducible bands generated, 29 (51.8%) were polymorphic. The band size ranged from 120 bp to 1100 bp. The polymorphic information content (PIC) value ranged from 0.29 to 0.76 with the average value 0.49. Patel *et al*. (2015) who studied nine ISSR primers out of the ten generated 71 bands/alleles of which 58 bands were polymorphic that was equal to 79.70% polymorphism and 13 bands were monomorphic with an average of 7.88 bands per primer. The highest PIC value of was noticed with HB-18 while lowest PIC value was recorded with I-28 with an average of 0.833 per primer. Sharma *et al*. (2017) worked on twenty ISSR primers out of 18 primers gave amplification. The Inter Simple Sequence Repeat (ISSR) research revealed a significant amount of polymorphism (85.71%). Using 18 ISSR primers, a total of 77 amplified bands were produced, with 67 of them being polymorphic. PIC values varied from 0.07 to 0.37, with an average of 0.300 seen across all varieties.

4.2.2.2 Identification of genotype specific marker from ISSR primers

Among all the ISSR primers, UBC-802 and UBC-806 primers showed the amplification of unique and variety specific bands (Table 4.6). UBC-806 primer produced one specific band with mol. wt. of 209 bp in GJG-HPS-1. On the other hand, UBC-802 primer produced two specific bands with mol. wt. of 1292 and 843 bp in the variety TG-37A.

4.2.2.3 Genetic similarity

The genetic similarity between each pair of 18 groundnut varieties was examined, and the findings indicated that TG-37A variety had the lowest similarity with GJG-17, GJG-22 and GG-6 with 33%, while GAUG-10 and GG-11 varieties had the maximum similarity of 92% (Table 4.7).

4.2.2.4 Cluster analysis of ISSR

Table 4.6: Molecular weight (bp) of varieties specific markers generated by ISSR primers

ISSR Primers	Varieties																	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
UBC-801	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-802	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1292
	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	843
UBC-803	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-805	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-806	-	-	-	-	-	209	-	-	-	-	-	-	-	-	-	-	-	-
UBC-810	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-811	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-812	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-813	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-848	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-852	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-857	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
UBC-862	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-	-	-	2

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

Table 4.7: Jaccard's similarity coefficient of groundnut varieties based on ISSR data analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	**0.92**	1.00																
3	0.84	0.86	1.00															
4	0.68	0.69	0.63	1.00														
5	0.77	0.70	0.71	0.69	1.00													
6	0.72	0.65	0.63	0.64	0.76	1.00												
7	0.81	0.74	0.71	0.61	0.77	0.76	1.00											
8	0.85	0.78	0.80	0.61	0.86	0.76	0.86	1.00										
9	0.73	0.66	0.76	0.61	0.77	0.69	0.73	0.77	1.00									
10	0.66	0.63	0.64	0.66	0.74	0.70	0.74	0.70	0.70	1.00								
11	0.78	0.72	0.70	0.71	0.83	0.85	0.79	0.79	0.83	0.80	1.00							
12	0.74	0.76	0.69	0.75	0.71	0.78	0.79	0.71	0.64	0.76	0.80	1.00						
13	0.70	0.67	0.64	0.51	0.63	0.81	0.70	0.74	0.67	0.57	0.76	0.64	1.00					
14	0.80	0.82	0.71	0.77	0.69	0.68	0.73	0.73	0.69	0.70	0.74	0.83	0.59	1.00				
15	0.55	0.51	0.52	0.57	0.63	0.66	0.49	0.56	0.63	0.52	0.69	0.50	0.63	0.55	1.00			
16	0.67	0.63	0.65	0.59	0.71	0.67	0.71	0.71	0.64	0.86	0.70	0.78	0.57	0.67	0.42	1.00		
17	0.69	0.62	0.68	0.57	0.70	0.65	0.70	0.70	0.78	0.67	0.72	0.67	0.59	0.73	0.59	0.68	1.00	
18	0.35	0.38	0.42	**0.33**	0.36	0.37	0.36	**0.33**	0.43	0.46	0.38	0.40	**0.33**	0.45	0.37	0.42	0.49	1.00

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

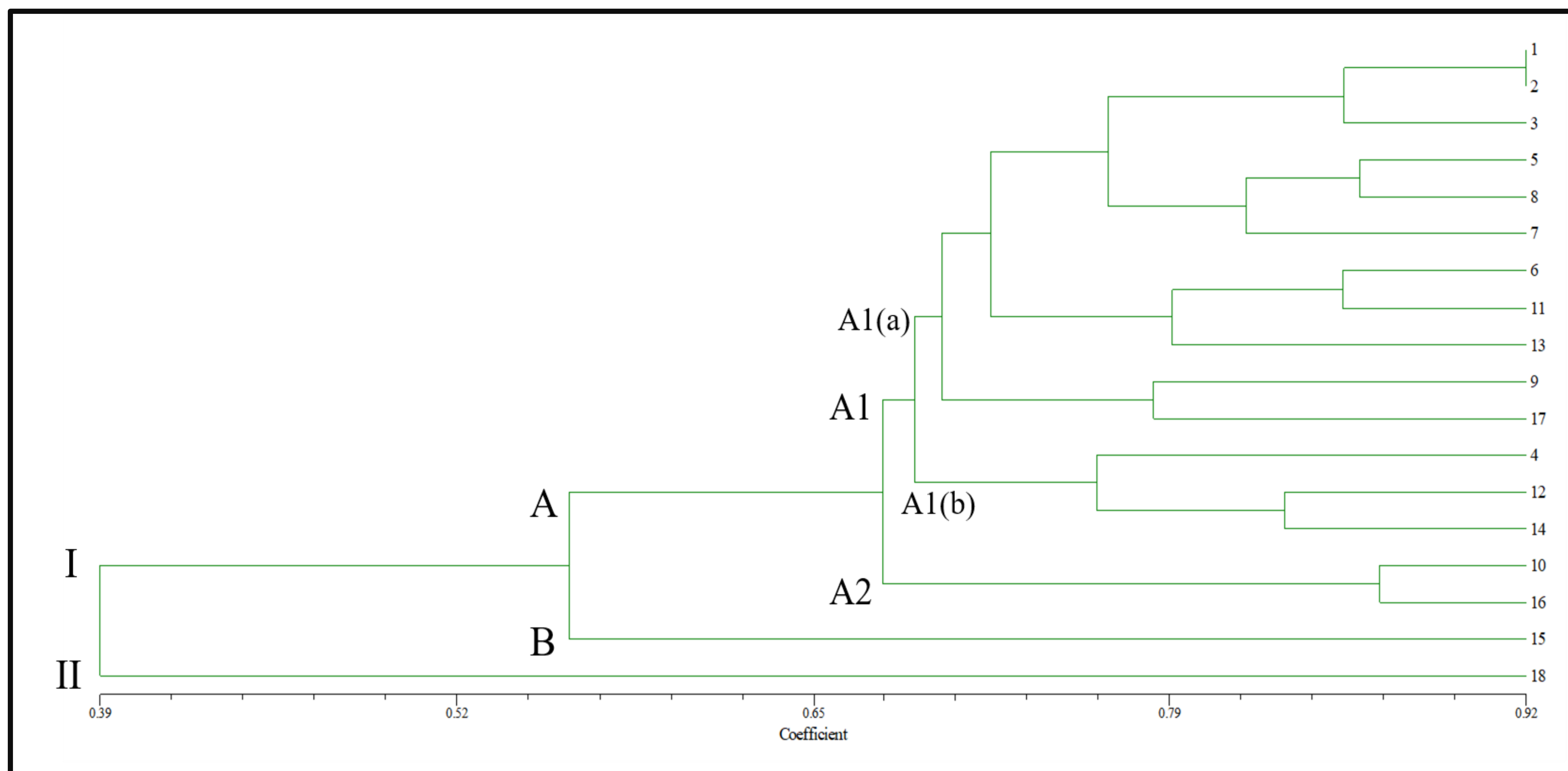
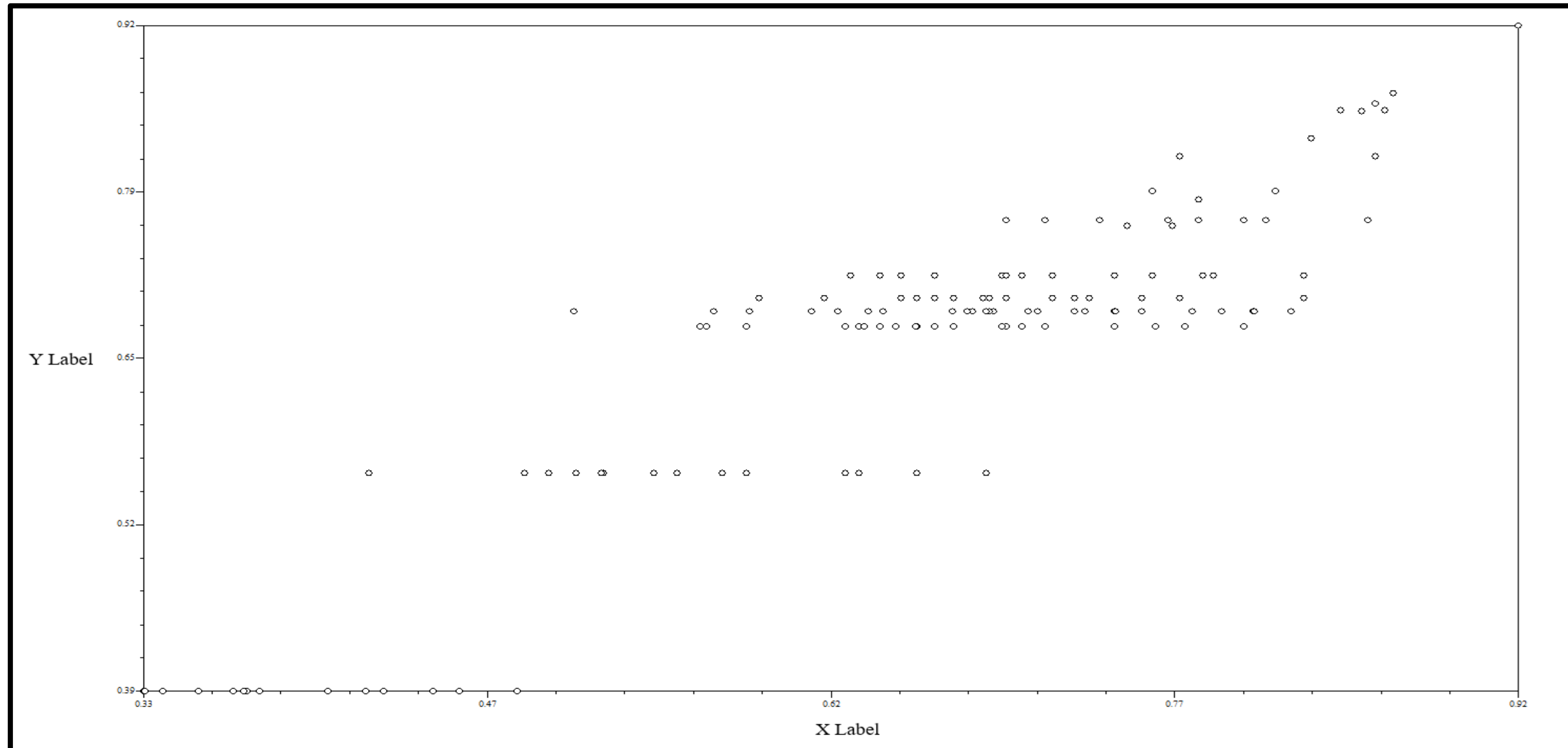


Fig. 4.12: Dendrogram depicting the genetic relationship among 18 groundnut varieties based on data of ISSR

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)



Matrix correlation: r = 0.89 (=Normalized Mantel statistic Z)

Fig. 4.13: Cophenetic values against Jaccard's similarity coefficients from ISSR data of 18 groundnut varieties

Similarity index and cluster analysis for ISSR data of eighteen groundnut varieties was done by Jaccard's coefficient and UPGMA using NTSYSpc-2.0 software. The groundnut varieties were grouped into two main clusters: clusters-I and II with an average similarity of 39% (Fig.4.12). Cluster-II consisted of only one variety TG-37A and was the most diverse among all the varieties.

The cluster-I comprised of two subclusters-A and B with 56% likeness. Subcluster A was further divided into group A1 and A2 having 67% relatedness. Group A1 was further divided into subgroups A1(a) and A1(b) having nearly 68% relatedness. Subgroup A1(a) consisted of eleven entries *viz*., GAUG-10, GG-11, GG-16, GG-41, GJG-22, GG-21, GJG-HPS-, KDG-128, GG-6, GG-HPS-2 and JL-501 while, subgroup A1(b) consisted of three varieties which were GJG-17, Kaushal and GG-7. Group A2 contained of only two variety KDG-123 and GJG-32 having nearly similarity 86%. Subcluster B consisted of only one genotype which was GJG-9.

To test the goodness of fit of the clustering of ISSR data (Fig. 4.13) and matrix of cophenetic values were also computed using the program COPH. The cophenetic matrixes were compared to the original matrixes produced by SIMQUAL. The plots of one matrix against the other and the association statistics were made and calculated by MAXCOMP. The plot and statistics of 18 groundnut varieties included in present study were shown in Figure 4.13. In the present investigation, the mental test statistics Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation r = 0.89) as categorized by Rohlf (1998) was found under the category of "**good fit".**

Similarly, Mohammed *et al*. (2022) worked on genetic variability and diversity of 43 accessions of *A. hypogaea* collected from different regions of Ethiopia using ISSR markers. Genetic relationship between *A. hypogaea* accessions based on Jaccard's pair wise similarity coefficients varied from 44 to 83% with an average value of 63.5%. The UPGMA analysis grouped *A. hypogaea* accessions into five distinct clusters at 63.5% similarity coefficient, and the principal coordinate analysis revealed similar grouping. Sharma *et al*. (2017) also worked on ISSR primers and found that Jaccard's similarity coefficient values varied from 0.54-0.90, with an average of 0.72. The majority of varieties could be split into five primary groups using the dendrogram created by the UPGMA algorithm and PCA. Clusters I, II and IV each had two genotypes, whereas Cluster III had nine genotypes. Cluster V had five genotypes.

4.2.3 Simple Sequence Repeat (SSR) Analysis

In the present study, for the characterization of groundnut varieties, microsatellite (SSR) markers were used with 18 groundnut varieties. Out of 61 SSR primers tested, 18 primers gave the amplification result.

4.2.3.1 Polymorphism pattern of SSR

All the 18 SSR primers were amplified a total of 37 bands. Out of 37 bands, 25 bands were polymorphic with an average of 1.39 bands per primer and 12 bands were monomorphic. Among the 25 polymorphic bands, 22 bands were shared polymorphic, while 3 bands were unique-polymorphic (Table 4.8). The amplified fragments having size ranged from 107-957 bp. The largest amplicon of 957 bp was amplified by SSR primer JAUGP-175 and the smallest fragment of 107 bp was found with JAUGP-197.

The per cent polymorphism obtained for SSR primers were ranged from 0 to 100% with an average value of 53.70% per primer. Out of 18 primers, 11 primers gave monomorphic fragments. The polymorphic information content (PIC) was calculated for each primer and it was ranged from 0.0 to 0.81 with an average value of 0.27 for each primer. The SSR primer index (SPI) differed from 0.0 to 4.86 with an average value of 0.94 (Table 4.8).

The performance of individual primer to amplify genomic DNA of 18 groundnut varieties is discussed as under.

JAUGP-100

Only one monomorphic fragment with a size of 195 bp was amplified by the primer JAUGP-100 and having 0.00% polymorphism and PIC value 0 (Table 4.8). This primer did not show any unique or shared polymorphic band.

JAUGP-101

The primer JAUGP-101 amplified one fragment with size of 147 bp and was monomorphic fragment having 0.00% polymorphism and PIC value 0 (Table 4.8). This primer did not generate any unique or shared polymorphic band on amplification.

JAUGP-103

The primer JAUGP-103 amplified only one fragment with size of 199 bp and it was having 100.00% polymorphism, shared polymorphic band and PIC value 0 (Table 4.8). This primer did not produce any unique or monomorphic band.

Table 4.8: Size, number of amplified bands, per cent polymorphism and PIC obtained by SSR primers in the 18 groundnut varieties

Sr. No.	SSR Primer	Band Size (bp)	Total No. of Bands (A)	Polymorphic Bands (B)			Mono-morphic Band	% Poly-morphism (B/A)	PIC	SPI
				S	U	T				
1	JAUGP-100	195	1	0	0	0	1	0.00	0.00	0.00
2	JAUGP-101	147	1	0	0	0	1	0.00	0.00	0.00
3	JAUGP-103	199	1	1	0	1	0	100.00	0.00	0.00
4	JAUGP-106	152	1	1	0	1	0	100.00	0.00	0.00
5	JAUGP-145	224	1	0	0	0	1	0.00	0.00	0.00
6	JAUGP-175	182-957	6	5	0	5	1	83.33	0.81	4.86
7	JAUGP-182	157-497	6	3	3	6	0	100.00	0.67	4.02
8	JAUGP-197	107-175	2	2	0	2	0	100.00	0.64	1.28
9	JAUGP-253	110	1	0	0	0	1	0.00	0.00	0.00
10	JAUGP-256	173-263	3	2	0	2	1	66.67	0.66	1.98
11	JAUGP-277	110-158	2	2	0	2	0	100.00	0.49	0.98
12	JAUGP-350	182-259	2	2	0	2	0	100.00	0.20	0.40
13	PM-36	241	1	1	0	1	0	100.00	0.00	0.00
14	PM-188	198-251	3	2	0	2	1	66.67	0.60	1.80
15	PM-238	147-161	2	0	0	0	2	0.00	0.50	1.00
16	PM-325	116	1	0	0	0	1	0.00	0.00	0.00
17	TC3H07	167	1	0	0	0	1	0.00	0.00	0.00
18	TC1E05	230-259	2	1	0	1	1	50.00	0.34	0.68
TOTAL			37	22	3	25	12	-	-	-
AVERAGE			-	-	-	1.39	0.67	53.70	0.27	0.94

S = Shared**; U** = Unique**; T** = Total polymorphic bands; **PIC** = Polymorphism information content; **IPI** = ISSR primer index = Number of bands x PIC

JAUGP-106

The primer JAUGP-106 amplified 1 fragment with a size of 152 bp and it was shared polymorphic fragment having 100.00% polymorphism and PIC value 0 (Table 4.8). This primer did not show any unique or monomorphic band.

JAUGP-145

One monomorphic fragment with a size of 224 bp was amplified by the primer JAUGP-145 and having 0.00% polymorphism and PIC value 0 (Table 4.8). This primer did not show any unique or shared polymorphic band.

JAUGP-175

Total 6 fragments were amplified by the primer JAUGP-175 with size ranging from182-957 bp. Among which 5 fragments were shared polymorphic and 1 fragment was monomorphic having 83.33% polymorphism and PIC value 0.81 (Table 4.8). This primer did not produce any unique band on amplification.

JAUGP-182

The primer JAUGP-182 amplified maximum number of 6 fragments among primer tested with size ranging from 157-497 bp. Among the 6 fragments, 3 shared band and 3 unique band were obtained having 100.00% polymorphism and PIC value 0.67 (Table 4.8). This primer did not produce any monomorphic band.

JAUGP-197

Amplified 2 fragments with size ranging from 107-175 bp were obtained by primer JAUGP-197 out of which all fragments were shared polymorphic having 100.00% polymorphism and PIC value 0.64 (Table 4.8). This primer did not give any unique or monomorphic band.

JAUGP-253

The primer JAUGP-253 amplified one fragment with size of 110 bp and was monomorphic fragment having 0.00% polymorphism and PIC value 0 (Table 4.8). This primer did not generate any unique or shared polymorphic band on amplification.

JAUGP-256

Total 3 fragments were amplified by the primer JAUGP-256 with size ranging from 173-263 bp. Among which 2 fragments were shared polymorphic and 1 fragment were monomorphic having 66.67% polymorphism and PIC value 0.66 (Table 4.8). This primer did not produce any unique band on amplification.

JAUGP-277

The primer JAUGP-277 amplified two fragment with a size ranging from 110-158 bp and both were shared polymorphic fragment having 100.00% polymorphism and PIC value 0.49 (Table 4.8). This primer did not show any unique or monomorphic band.

JAUGP-350

The primer JAUGP-350 amplified 2 fragments with size ranged between 189-259 bp and they were shared polymorphic band having 100.00% polymorphism and PIC value 0.20 (Table 4.8). This primer did not produce any unique or monomorphic band.

PM-36

On amplification, the primer PM-36 amplified only one fragment of 241 bp size and it was shared polymorphic fragment having 100.00% polymorphism and PIC value 0 (Table 4.8). This primer did not amplify any unique or monomorphic band.

PM-188

Total 3 fragments were amplified by the primer PM-188 with size ranging from 198-251 bp, from which 2 fragments were shared polymorphic and 1 fragment was monomorphic having 66.67% polymorphism and PIC value 0.60. This primer did not show any unique band (Table 4.8).

PM-238

The primer PM-238 produced two fragments with size ranged between 147-161 bp and both were monomorphic fragment having 0.00% polymorphism and PIC value 0.50 (Table 4.8). This primer did not produce any unique or shared polymorphic band.

PM-325

The primer PM-325 amplified only 1 fragment with size of 116 bp and it was monomorphic band having 0.00% polymorphism and PIC value 0 (Table 4.8). This primer did not produce any unique or polymorphic band.

TC3H07

One monomorphic fragment with a size of 167 bp was amplified by the primer TC3H07 and having 0.00% polymorphism and PIC value 0 (Table 4.8). This primer did not show any unique or shared polymorphic band.

TC1E05

The primer TC1E05 gave two fragments with size of 230 bp and 259 bp, from which 1 fragment was shared polymorphic and 1 fragment was monomorphic having

50.00% polymorphism and PIC value 0.34 (Table 4.8). This primer did not produce any unique band.

Similar results are reported by Kalyani and Sasidharan (2021) who analyzed a total of 1293 scorable bands with total 251 alleles were found in a pooled SSR analysis of 50 groundnut varieties using 23 SSR primers. The PIC value on average was 0.670. Hong *et al*. (2021) also did molecular characterization with 40 highly polymorphic simple sequence repeats (SSRs) yielded a total of 167 alleles, with an average of 4.18 alleles per marker. These markers polymorphism information content (PIC) ranged from 0.79 to 0.26, with an average value of 0.55 per marker.

Similarly, Wang *et al*. (2015) developed highly informative simple sequence repeat (SSR) markers to assess the genetic diversity and population structure of peanut cultivars and breeding lines from different breeding programs in China, India and the US. For this study, 111 SSR markers were chosen, yielded a total of 472 alleles. Gene diversity and polymorphic information content (PIC) had mean values of 0.480 and 0.429, respectively. Bhad *et al*. (2016) involved in the development of 192 SSR markers from Arachis genomic survey sequences. These polymorphic SSR markers amplified 155 bands (76 genomic and 79 genic), 128 of which were polymorphic (67 genomic and 61 genic). Allelic polymorphism was found 88.1% of genomic SSRs and 77.2% of genic SSRs. The markers polymorphic information content (PIC) ranged from 0.04 to 0.95. Gaikpa *et al*. (2015) also discovered molecular diversity among cultivated groundnut using SSR markers for further improvement. All 13 SSR markers were polymorphic, with PIC values over 0.50 in 76.9% of them. Genetic similarities between the 20 varieties ranged from 60 to 90%, according to clustering analyses.

4.2.3.2 Identification of genotype specific marker from SSR primers

Among all SSR primers, only JAUGP-182 primer showed the amplification of unique and genotype specific bands (Table 4.9). Primer JAUGP-182 produced three specific bands with mol. wt. of 417 bp in GJG-HPS-1, while two bands of size 392 bp and 497 bp were in KDG-128 varieties.

4.2.3.3 Genetic similarity

Genetic similarity was determined for each pair of eighteen groundnut varieties which revealed that the lowest similarity of 56% was noticed between KDG-123 and GG-6, while highest of 94% was observed between GG-41 with GJG-HPS-1 and TG-37A, and GJG-17 with GG-41. Overall GG-41 had the maximum similarity with GJG-

Table 4.9: Molecular weight (bp) of varieties specific markers generated by SSR primers

SSR Primers	Varieties and Varieties																	
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
JAUGP 100	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 106	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 145	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 175	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 182	-	-	-	-	-	-	-	-	-	-	392	-	-	-	-	-	-	-
	-	-	-	-	-	417	-	-	-	-	-	-	-	-	-	-	-	-
	-	-	-	-	-	-	-	-	-	-	497	-	-	-	-	-	-	-
JAUGP 197	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 253	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 256	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 277	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
JAUGP 350	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PM-36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PM-188	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PM-238	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
PM-325	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TC3H07	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
TC1E05	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Total	-	-	-	-	-	1	-	-	-	-	2	-	-	-	-	-	-	-

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

Table 4.10: Jaccard's similarity coefficient of groundnut varieties based on SSR data analysis

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	0.88	1.00																
3	0.79	0.82	1.00															
4	0.83	0.86	0.90	1.00														
5	0.77	0.81	0.84	**0.94**	1.00													
6	0.72	0.75	0.84	0.88	**0.94**	1.00												
7	0.61	0.65	0.68	0.72	0.73	0.73	1.00											
8	0.68	0.71	0.69	0.78	0.84	0.79	0.80	1.00										
9	0.81	0.78	0.75	0.79	0.74	0.74	0.63	0.65	1.00									
10	0.79	0.76	0.79	0.90	0.84	0.78	0.68	0.74	0.69	1.00								
11	0.73	0.71	0.74	0.73	0.74	0.74	0.64	0.65	0.76	0.64	1.00							
12	0.75	0.72	0.76	0.80	0.75	0.75	0.65	0.71	0.78	0.70	0.77	1.00						
13	0.66	0.63	0.61	0.66	0.67	0.67	0.72	0.68	0.74	**056**	0.58	0.69	1.00					
14	0.76	0.73	0.83	0.81	0.76	0.76	0.77	0.72	0.67	0.77	0.67	0.68	0.70	1.00				
15	0.68	0.71	0.74	0.78	0.79	0.79	0.80	0.87	0.70	0.69	0.65	0.77	0.79	0.83	1.00			
16	0.78	0.81	0.79	0.83	0.77	0.77	0.67	0.73	0.81	0.72	0.68	0.88	0.71	0.70	0.79	1.00		
17	0.76	0.79	0.77	0.87	0.81	0.76	0.66	0.67	0.79	0.77	0.62	0.73	0.76	0.74	0.72	0.82	1.00	
18	0.77	0.80	0.83	0.93	**0.94**	0.88	0.67	0.78	0.73	0.83	0.68	0.80	0.71	0.75	0.78	0.83	0.87	1.00

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

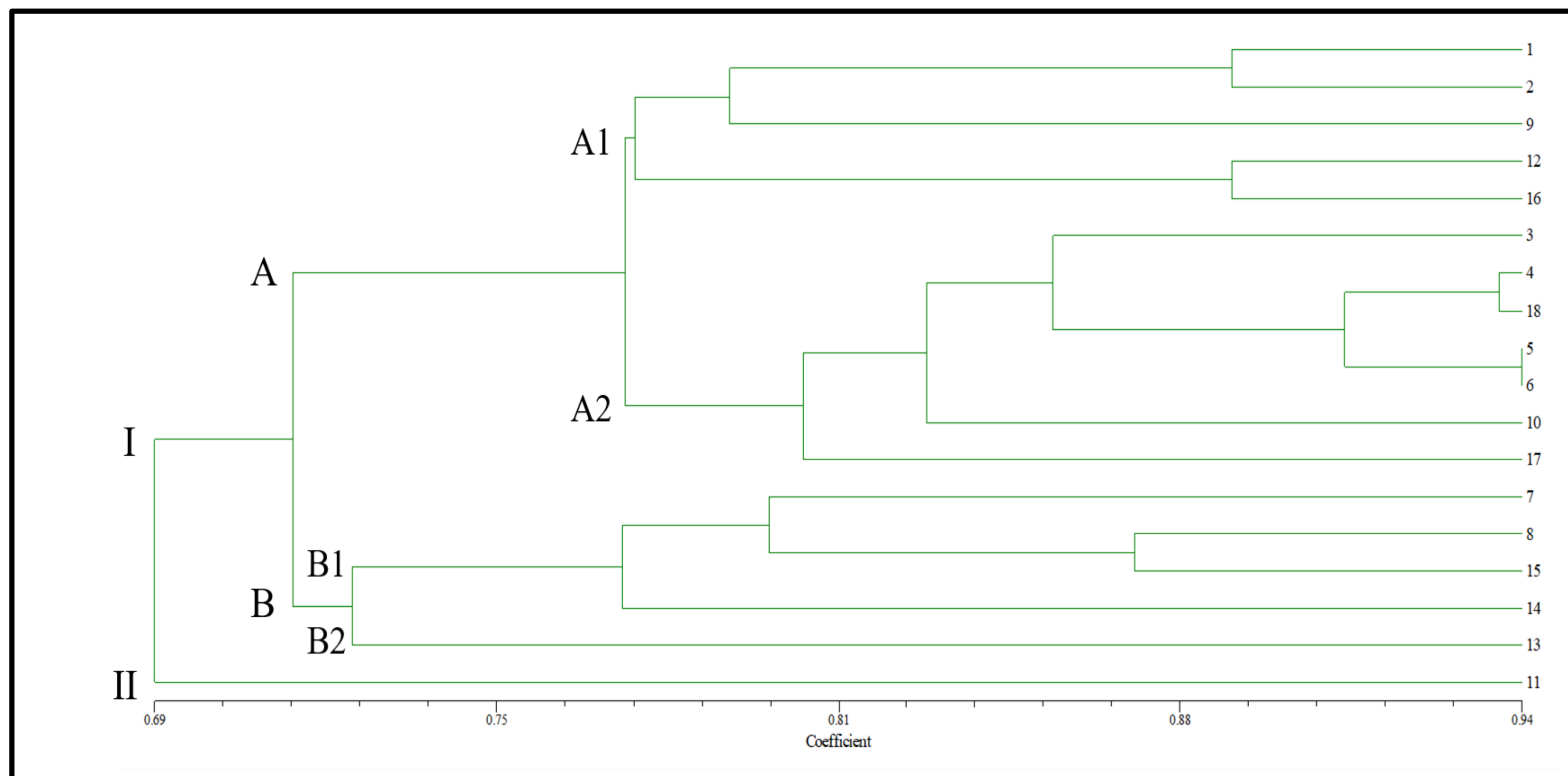
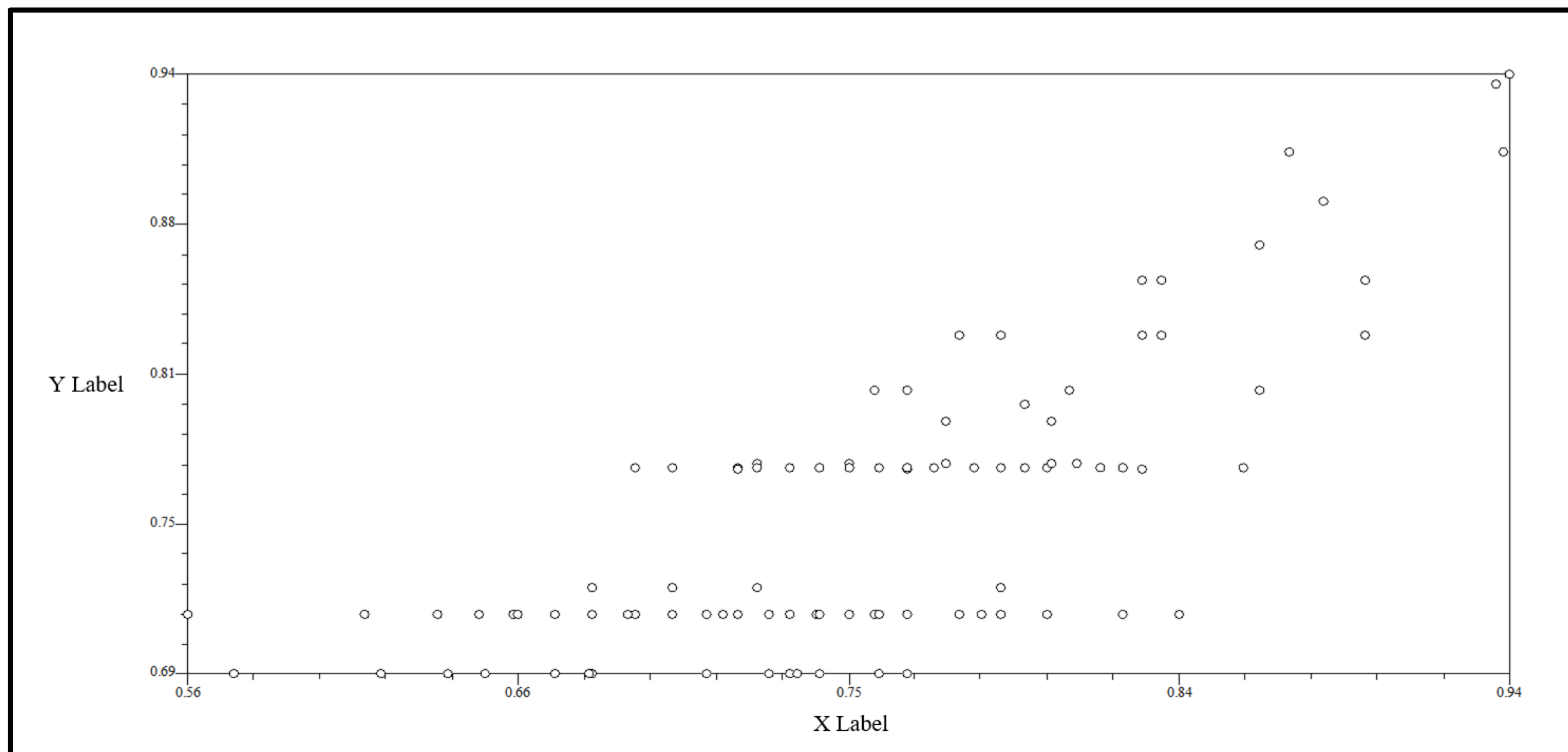


Fig. 4.14: Dendrogram depicting the genetic relationship among 18 groundnut varieties based on SSR markers
(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)



Matrix correlation: r = 0.76 (=Normalized Mantel statistic Z)

Fig. 4.15: Cophenetic values against Jaccard's similarity coefficients from SSR data of 18 groundnut varieties

HPS-1, GJG-17 ang TG-37A (Table 4.10).

4.2.3.4 Cluster Analysis of SSR

The dendrogram was constructed using UPGMA based on Jaccard's similarity coefficient through NTSYSpc-2.02i software for SSR data of eighteen varieties (Table 4.10 and Fig. 4.14). The 18 groundnut varieties were grouped into two main clusters: cluster-I and cluster-II sharing 69% similarity. The cluster-I was divided into two subcluster-A and B both contained a total of 17 varieties (Fig. 4.14). Subcluster-A was further bifurcated into two groups A1 and A2 having nearly 77% likeness. Group A1 consisted of only five variety GAUG-10, GG-11, GG-HPS-2, Kaushal and GJG-32, while group A2 consisted of seven entries *viz*., GG-16, GJG-17, TG-37A, GG-41, GJG-HPS-1 and KDG-123 having nearly 80% similarity. Subcluster-B was further bifurcated into two groups B1 and B2 having nearly 73% likeness. Group B1 consisted of four variety such as GG-21, GJG-22, GJG-9 and GG-7, while group B2 consisted of only one variety GG-6. The cluster-II consisted of only one variety KDG-128 were the most diverse among all eighteen varieties.

To test the goodness of fit of the clustering of SSR data, matrix of cophenetic values were also computed using the program COPH. The cophenetic matrices were compared to the original matrices produced by SIMQUAL. The plots of one matrix against the other and the association statistics were made and calculated by MAXCOMP. The plot and statistics of eighteen groundnut varieties included in present study were shown in Figure 4.15. In the present investigation the mental test statistics Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation r = 0.76) as categorized by Rohlf (1998) was found under the category of **"poor fit".**

Similarly**,** Islam *et al*. (2008) utilized groundnut microsatellite markers in 23 elite groundnut genotypes to assess the genetic diversity. The UPGMA dendrogram divided the aforementioned groundnut genotypes into two primary groups (I and II). Cluster I had two sub-clusters la and lb and cluster II consisted of two genotypes namely, ICGV 94165 and ICGV 00340 were unique and diversed from all other genotypes belonging to cluster I. Gautami *et al*. (2009) also investigated the germplasm analysis and interspecific transferability of a novel collection of groundnut SSR markers. Peanut germplasm was separated into four clusters by the dendrogram. Clusters I and II are made up entirely of cultivated tetraploid accessions, Cluster III is made up entirely of amphidiploids, and Cluster IV is made up entirely of wild (diploid)

accessions. Except for IAC-R886, which is a Brazilian accession, Cluster I consisted of eight cultivated tetraploid accessions from India.

4.2.4 Pooled study of molecular markers:

Groundnut varieties may be separated from one another to some extent using molecular markers such as RAPD, ISSR and SSRs. The reliability of one particular marker does not fulfill the goal of identification of varieties. As a result, a pooled cluster analysis including three molecular markers was performed to evaluate the differences and similarities across varieties and varieties found in this study.

4.2.4.1 Genetic similarity

The genetic similarity of all three molecular markers was assessed for each pair of eighteen groundnut varieties, revealing that GAUG-10 and GG-11 had the highest similarity of 87%, while TG-37A with GJG-HPS-1, KDG-128 and GG-6 had the minimum similarity of 61% (Table 4.11).

4.2.4.2 Cluster Analysis of RAPD, ISSR and SSR

Jaccard's similarity coefficient and UPGMA method were used to develop a dendrogram (Table 4.11 and Fig 4.16) which divided the varieties into two main clusters I and II with an average resemblance of 66% (Fig 4.16). Cluster-I was divided into two subclusters A and B with nearly 67% similarity. Subcluster A was divided into two groups A1 and A2 with nearly 71% similarity. Group A1 was further bifurcated into two groups A1(a) and A1(b) having nearly 73% likeness. Group A1(a) consisted of all twelve varieties such as GAUG-10, GG-11, GJG-17, GG-41, GJG-22, KDG-128, Kaushal, GG-7, GJG-32, KDG-123, GJG-HPS-1 and GG-6 while, group A1(b) consisted of two varieties GG-16 and GG-21. Subcluster A2 consisted of one varieties GJG-9. Subcluster B consisted of only one varieties GG-HPS-2.

The cluster-II consisted of two varieties such as JL-501 and TG-37A and were the most diverse among all the eighteen varieties.

Similarly, the correlation among RAPD, ISSR and SSR data, matrix of cophenetic values were computed using the tree matrix produced by SAHN to calculate the cophenetic values by the program COPH. The Mantel's test statistics Z was normalized and degree of fit for cluster analysis (Matrix correlation r =0.757). RAPD, ISSR and SSR data were combined for UPGMA cluster analysis of them which allowed

for two main cluster. Jaccard's similarity coefficient ranged from 0.437 to 0.725 (Patel *et al*., 2015).

To test the goodness of fit of the clustering of RAPD, ISSR and SSRs data, matrices of cophenetic values were also computed using the program COPH (Fig 4.8). In the present study also the mental test statistics Z was normalized and degree of goodness of fit for a cluster analysis (Matrix correlation r = 0.83) as categorized by Rohlf (1998) was found under the category of **"good fit".**

Table 4.11: Jaccard's similarity coefficient of 18 groundnut varieties based on pooled data of molecular markers

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
1	1.00																	
2	**0.87**	1.00																
3	0.78	0.75	1.00															
4	0.82	0.80	0.75	1.00														
5	0.83	0.77	0.75	0.86	1.00													
6	0.75	0.69	0.72	0.78	0.82	1.00												
7	0.80	0.75	0.76	0.75	0.78	0.73	1.00											
8	0.79	0.73	0.70	0.78	0.83	0.78	0.78	1.00										
9	0.72	0.63	0.65	0.69	0.71	0.64	0.64	0.69	1.00									
10	0.74	0.70	0.65	0.79	0.79	0.74	0.70	0.76	0.65	1.00								
11	0.82	0.73	0.69	0.79	0.81	0.79	0.74	0.78	0.76	0.76	1.00							
12	0.78	0.73	0.68	0.82	0.79	0.78	0.73	0.79	0.68	0.78	0.85	1.00						
13	0.74	0.68	0.65	0.73	0.73	0.80	0.73	0.79	0.65	0.71	0.78	0.79	1.00					
14	0.80	0.76	0.71	0.84	0.78	0.75	0.75	0.78	0.66	0.75	0.81	0.82	0.78	1.00				
15	0.72	0.65	0.65	0.75	0.72	0.72	0.69	0.73	0.67	0.67	0.77	0.71	0.76	0.77	1.00			
16	0.78	0.73	0.68	0.78	0.79	0.73	0.74	0.77	0.66	0.77	0.75	0.80	0.72	0.76	0.69	1.00		
17	0.72	0.72	0.65	0.70	0.71	0.67	0.67	0.68	0.64	0.66	0.66	0.68	0.66	0.69	0.64	0.70	1.00	
18	0.67	0.63	0.65	0.69	0.68	0.65	0.63	0.64	**0.61**	0.64	**0.61**	0.66	**0.61**	0.66	0.63	0.70	0.72	1.00

(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

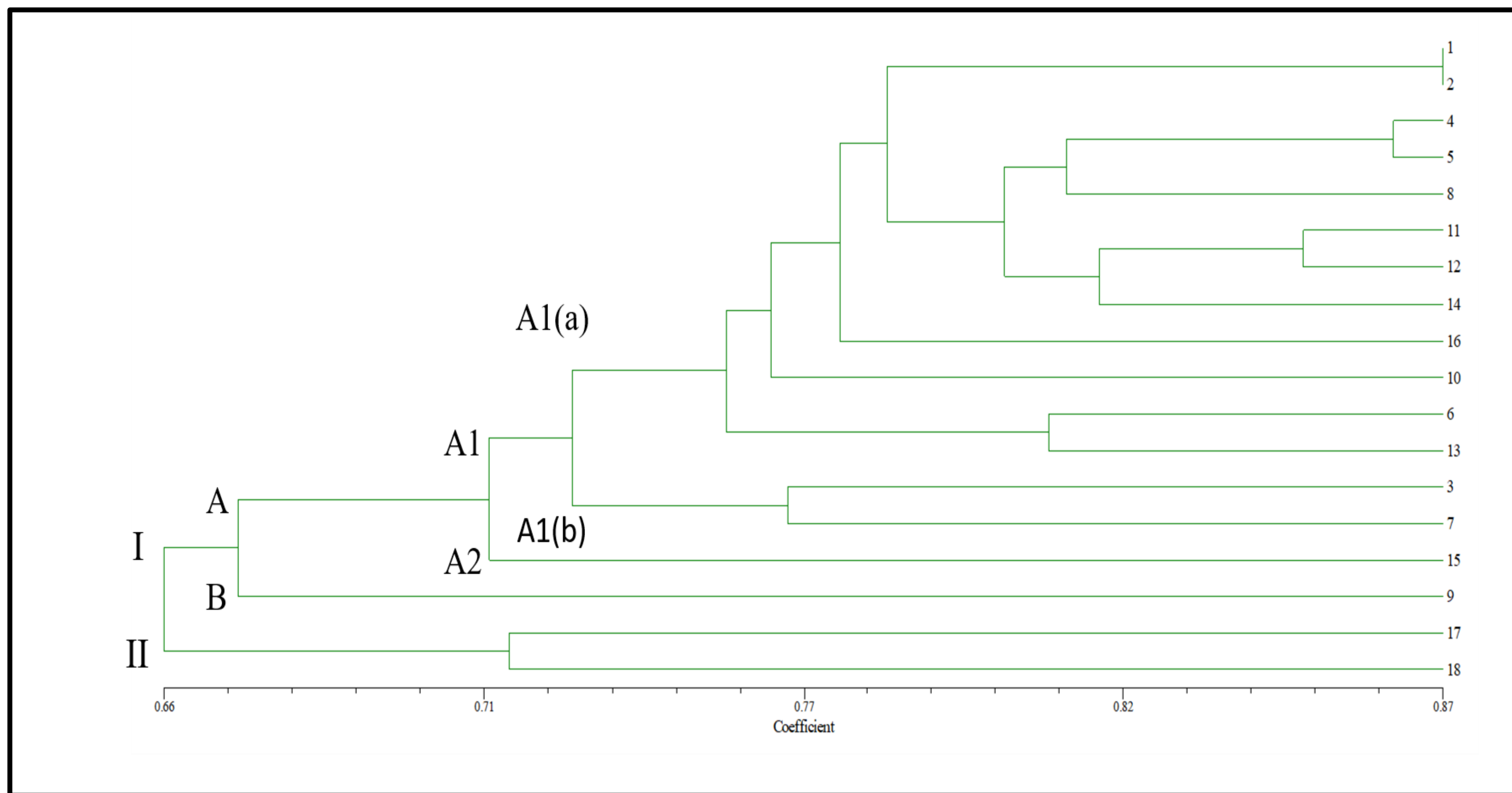
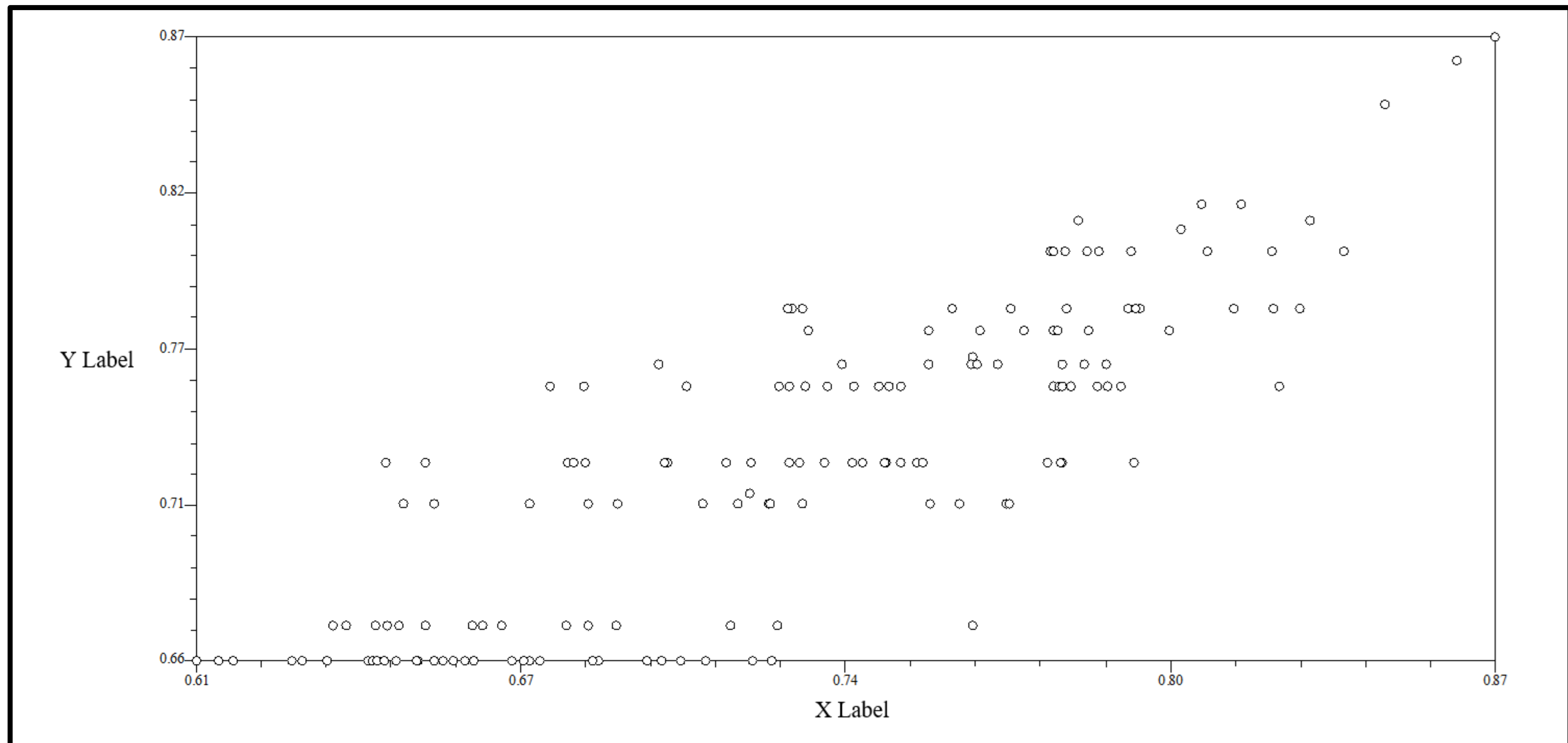


Fig. 4.16: Dendrogram depicting the genetic relationship among 18 groundnut varieties based on pooled data of molecular markers
(1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)



Matrix correlation: r = 0.83 (=Normalized Mantel statistic Z)

Fig. 4.17: Cophenetic values against Jaccard's similarity coefficients from pooled data of 18 groundnut varieties

CHAPTER - V

SUMMARY AND CONCLUSIONS

The experiment entitled **'Genetic diversity analysis in groundnut (*Arachis hypogaea* L.) based on molecular markers and biochemical parameters**' was conducted at Department Biotechnology, Junagadh Agricultural University, Junagadh during 2021-22 in the laboratory. In this chapter, the results are summarized and concluded. The experimental material consisted of 18 different groundnut varieties that were analyzed for biochemical and molecular analysis.

DNA-based molecular marker techniques such as RAPD, ISSR and SSR are independent of environmental conditions, providing significant advantages in species identification in terms of speed, cost, and elimination of the requirement for a grow out test. The current experiment was planned and carried out with the following three major objectives.

1. To analyse the groundnut varieties for the biochemical parameters.
2. To assess the groundnut varieties for genetic diversity using molecular markers.
3. To find out the phylogenic relationship among different groundnut varieties.

5.1 BIOCHEMICAL PARAMETERS

The eighteen varieties consisting 6 from each spreading, semi-spreading and bunch group were selected for the study. The result on biochemical parameters showed that all the parameters studied were varied significantly among the varieties. Moisture content was maximum in the variety GG-41 with 9.12% and the minimum was in KDG-123 with 5.59%. The highest oil content was recorded in the GJG-32 with 53.56%, while the minimum was in the variety Kaushal with 46.16%. The total carbohydrate content was recorded maximum 22.96% in variety GG-HPS-2, while minimum 14.49% was in the variety GJG-32. The lowest total protein and true protein values were found in the varieties GG-21 and JL-501 with 19.24% and 17.40%, respectively, while the highest total protein and true protein content with 24.84% and 20.98% observed in the variety KDG-123, respectively. The soluble sugar content ranged between 7.38% in the GJG-32 to 11.95% in the GG-HPS-2. The highest value of reducing sugar content observed in the variety TG-37A with 3.05%, while the lowest value of reducing sugar content recorded in the variety GJG-22 with 1.67%. Non-reducing sugar content was

maximum in the variety GG-HPS-2 with 9.56%, while the minimum value was 4.87% with the variety GJG-32. The lowest ash content 1.64% was observed in the GG-41 variety and the highest 3.27% was in the variety GJG-9.

5.2 MOLECULAR CHARACTERIZATION

Groundnut seedling leaves were used to extract whole genomic DNA. The CTAB technique was used to extract DNA, with some minor alterations. The DNA was spectrophotometrically measured to estimate DNA concentration in order to do PCR-based analysis. The concentration varied from 158.7 to 1189.2 ng/l, and the quality was determined by measuring the absorbance ratio of DNA at A260/A280, which was ranged from 1.40 to 2.06. RAPD, ISSR and SSR markers were utilized to study genetic diversity among the 18 groundnut varieties in this research.

5.2.1 RAPD Analysis

A total of 21 RAPD primers were amplified which produced the total 118 bands, including 20 monomorphic bands and 98 polymorphic bands, with 91 shared and 7 unique bands having an average of 4.7 bands per primer. The Polymorphism Information Content (PIC) values for RAPD markers ranged from 0 (CMN-A44) to 0.95 (CMN-A01), with an average value of 0.78 per primer, while the RAPD primer index (RPI) varied from 0 (CMN-A44) to 9.68 (OPN-10), with an average value of 4.64. Out of 21 RAPD primers, OPA-16, OPL-01, OPN-10 and OPZ-19 primers were able to produce variety specific unique bands. The UPGMA method produced two primary clusters in the phylogenetic tree, with similarity coefficients ranging from 54 to 91%. The varieties GG-HPS-2 and JL-501 had the lowest similarity of 54%, while GJG-17 and GG-41 varieties had the maximum similarity of 91%. Cluster analysis of RAPD showed that between cluster-I and cluster-II, 17 varieties were in cluster-I, while cluster-II only one variety GG-HPS-2 which was the most diverse among all.

5.2.2 ISSR Analysis

A total of 13 ISSR primers were amplified which generated 51 fragments from which 43 bands were polymorphic having 40 shared and 3 unique bands with an average of 3.30 bands per primer. The Polymorphism Information Content (PIC) values for ISSR marker were ranged from 0.00 (UBC-848) to 0.87 (UBC-806) with an average value of 0.63 per primer and ISSR primer index (IPI) varied from 0 (UBC-848) to 6.96

(UBC-806) with an average value of 2.79. Out of 13 ISSR primers, 2 primers were able to produce unique bands *viz*. UBC-802 and UBC-806. UBC-806 primer produced one variety specific band with mol. wt. of 209 bp in GJG-HPS-1, while UBC-802 primer produced two specific bands with mol. wt. of 1292 bp and 843 bp in TG-37A. The UPGMA method generated two main clusters in the phylogenetic tree, with similarity coefficients ranging from 33 to 92%. The varieties TG-37A with GJG-17, GJG-22, and GG-6 had the minimum similarity with 33%, whereas the varieties GAUG-10 and GG-11 had the highest similarity with 92%. Result of cluster analysis of ISSR showed that in cluster-I and cluster-II. Cluster-I had only one variety TG-37A which was the most diverse among all.

5.2.3 SSR Analysis

Eighteen SSR primers were used for molecular study of 18 groundnut varieties which produced the total 37 fragments among which 25 fragments were polymorphic 22 shared polymorphic and 3 unique with an average of 1.39 band per primer was generated. The Polymorphism Information Content (PIC) values for SSR markers were ranged from 0.0 (JAUGP-100, JAUGP-101, JAUGP-103, JAUGP-106, JAUGP-145, JAUGP-253, PM-36, PM-325 and TC3H07) to 0.81 (JAUGP-175) with an average value of 0.27 per primer and SSR primer index (SPI) differed from 0.00 (JAUGP-100, JAUGP-101, JAUGP-103, JAUGP-106, JAUGP-145, JAUGP-253, PM-36, PM-325 and TC3H07) to 4.86 (JAUGP-175) with an average value of 0.94. The phylogenetic tree constructed by UPGMA method generated two main clusters and similarity coefficient was ranged from 56 to 94%. The lowest similarity of 56% was noticed between KDG-123 and GG-6, while the highest of 94% was observed between GG-41 with GJG-HPS-1 and TG-37A and between GJG-17 with GG-41.

The pooled data of RAPD, ISSR and SSR markers used to generate mixed clustering pattern to confirm the difference and similarity between groundnut entries as the reliability of one particular marker does not fulfill the goal of identification of different groundnut varieties. The phylogenetic tree constructed by UPGMA method generated two main clusters and similarity coefficient was ranged from 87% among TG-37A with GG-HPS-1, KDG-128 and GG-6 to 61% between GAUG-10 and GG-11.

Conclusion

Total 9 biochemical parameters involved in this study. A wide range of variations were observed in different biochemical parameters could be due to different types of groundnut varieties. The 18 groundnut entries were selected for the study in which moisture content was maximum in the variety GG-41 with 9.12% and the minimum was in KDG-123 with 5.59%. The highest oil content was recorded in the GJG-32 variety with 53.56%, while the minimum was in the variety Kaushal with 46.16%. The total carbohydrate was recorded maximum in GG-HPS-2 variety with 22.96%, while the lowest was in the GJG-32 with 14.49%. The lowest value for total protein and true protein were observed in the variety GG-21 and JL-501 with 19.24% and 17.40%, respectively, while the highest value 24.84% of total protein and 20.98% of true protein was observed in the variety KDG-123. The soluble sugar content ranged between 7.38% in variety GJG-32 to 11.95% in the variety GG-HPS-2. The highest value of reducing sugar content observed in variety TG-37A with 3.05%, while the lowest value of reducing sugar content recorded in the variety GJG-22 with 1.67%. Maximum non-reducing sugar content was 9.56% in the variety GG-HPS-2, while the minimum value was 4.87% with the variety GJG-32. The lowest ash content 1.64% was recorded in the GG-41 variety and the highest 3.27% was in the variety GJG-9.

Based on the molecular markers associated with groundnut varieties, it could be concluded that molecular markers like RAPD and ISSR are most reliable to distinguish groundnut varieties. RAPD primers OPA-16, OPL-01, OPN-10 and OPZ-19 and ISSR primers UBC-802 and UBC-806 gave varieties specific unique band in a groundnut varieties. Among the molecular markers, SSR gave better similarity coefficient in the range 56% to 94%, compared to ISSR which had 33% to 92% similarity coefficient. Therefore, from the present experiment, the data generated might be useful to some extent for the identification of the genetic diversity among all the groundnut varieties.

The phylogenetic tree constructed by UPGMA method generated two main clusters in RAPD, ISSR, SSR and pooled data. In RAPD, similarity coefficient was ranged from 54 to 91%. The lowest genetic similarity of 54% was noticed between GG-HPS-2 with JL-501, while the highest was 91% noticed between GJG-17 and GG-41 varieties. In ISSR, similarity coefficient was ranged between 33 to 92%. Varieties GAUG-10 and GG-11 showed maximum similarity with 92% and the lowest similarity was 33% between the varieties TG-37A with GJG-17, GJG-22 and GG-6. In SSR,

similarity coefficient was ranged from 56 to 94%. The lowest similarity with 56% was noticed among KDG-123 with GG-6, while the highest of 94% similarity was observed between GG-41 with GJG-HPS-1 and TG-37A and between GJG-17 with GG-41. In pooled data of RAPD, ISSR and SSR, similarity coefficient was ranged from 61% between TG-37A with GG-HPS-2, KDG-128 and GG-6 to 87% between GAUG-10 and GG-11.

Overall conclusion of the study showed that there were wide variations in the biochemical composition of the groundnut varieties might be due to different types. The variety GJG-32 was found with the highest oil content and minimum in total carbohydrate, total soluble sugar and non-reducing sugar content and found unique among all.

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Not only this....

But my whole life is

Dedicated to

My Beloved

Parents....

Janki....

LIST OF ABBREVIATIONS

Abbreviations		Full Name
µl	:	Microlitre
µg	:	Microgram
Bp	:	Base-Pairs
BPB	:	Bromophenol Blue
C. V.	:	Co-efficient of variance
COPH	:	Cophenetic Values
CRD	:	Completely Randomized Design
CTAB	:	Cetyl Di Methyl Ethyl Ammonium Bromide
dNTPs	:	Deoxynucleotide Triphosphate
EDTA	:	Ethylene Diamine Tetra Acetic acid
EtBr	:	Ethidium Bromide
et al.	:	Co- Workers
gm	:	Gram
GC	:	Guanine- Cytosine
Ha	:	Hector
HCl	:	Hydrochloric Acid
IPI	:	ISSR Primer Index
IPR	:	Intellectual Property Rights
ISSR	:	Inter Simple Sequence Repeat
M	:	Molar
MAXCOMP	:	Matrix Comparison
mg	:	Milligram
ml	:	Milliliter
MW	:	Molecular Weight
NaCl	:	Sodium Chloride
NaOH	:	Sodium Hydroxide

nm	:	Nanometre
NTSYS	:	Numerical Taxonomy and Multivariate AnalysisSystem
OD	:	Optical Density
PCR	:	Polymerase Chain Reaction
pH	:	Potential of Hydrogen
PIC	:	Polymorphic Information Content
pMol	:	Pico Moles
ppm	:	Parts Per Million
PVP	:	Polyvinyl Pyrolidone
RAPD	:	Random Amplified Polymorphic DNA
Rf		Relative Fertility
RPI	:	RAPD Primer Index
rpm	:	Revolution Per Minute
SAHN	:	Sequential, Agglomerative, Hierarchical and Nested ClusteringvMethod
S. Em.	:	Standard error of mean
SI	:	Similarity index
SIMQUAL	:	Similarity for Qualitative Data
SPI	:	SSR Primer Index
SSR	:	Simple Sequence Repeats
Taq	:	*Thermus Aquaticus*
TBE	:	Tris-borate EDTA
TE	:	Tris- EDTA`
Tm	:	Melting Temperature
UPGMA	:	Unweighted Pair-Group Method with Arithmetic Averages
UV	:	Ultra-Violet

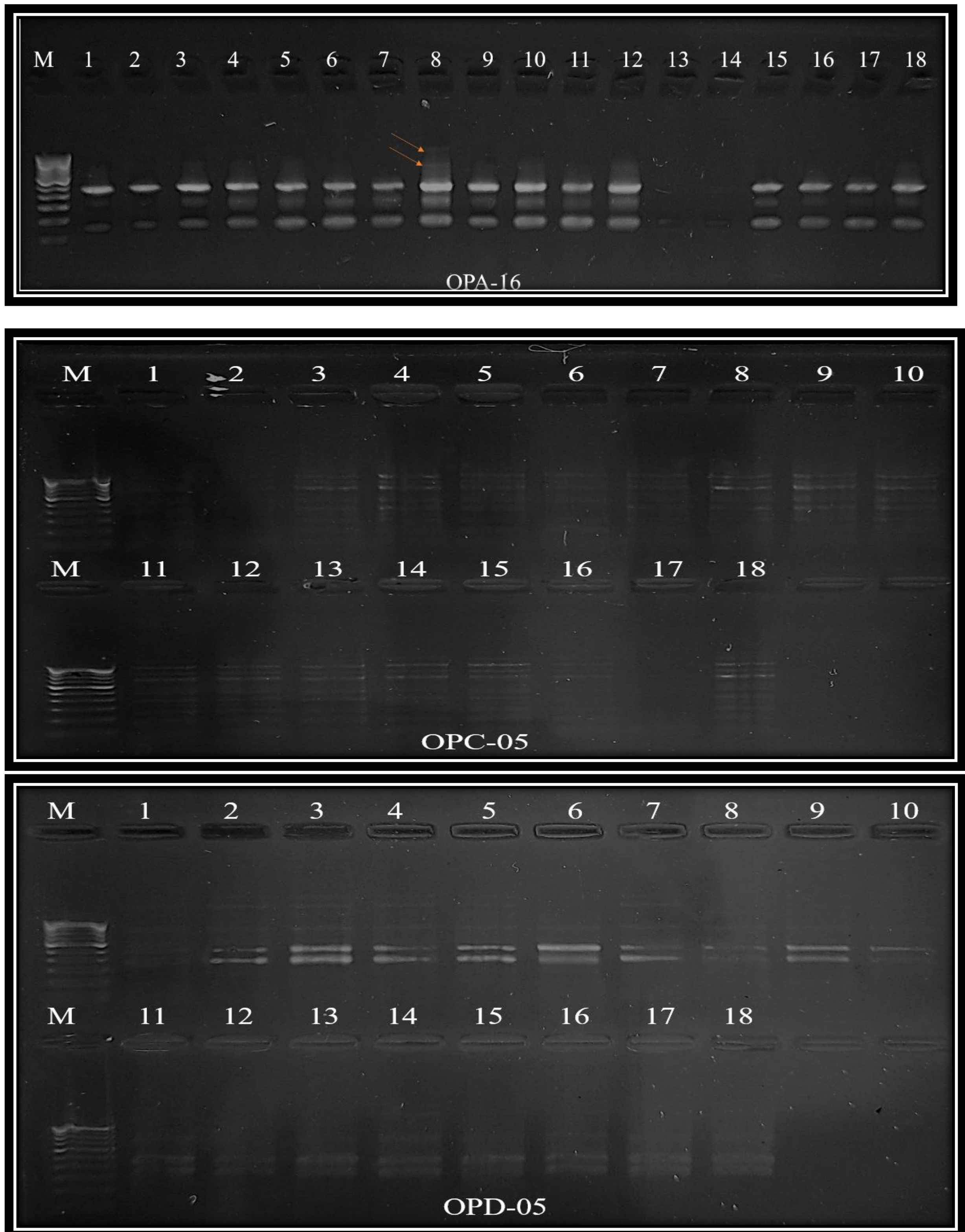
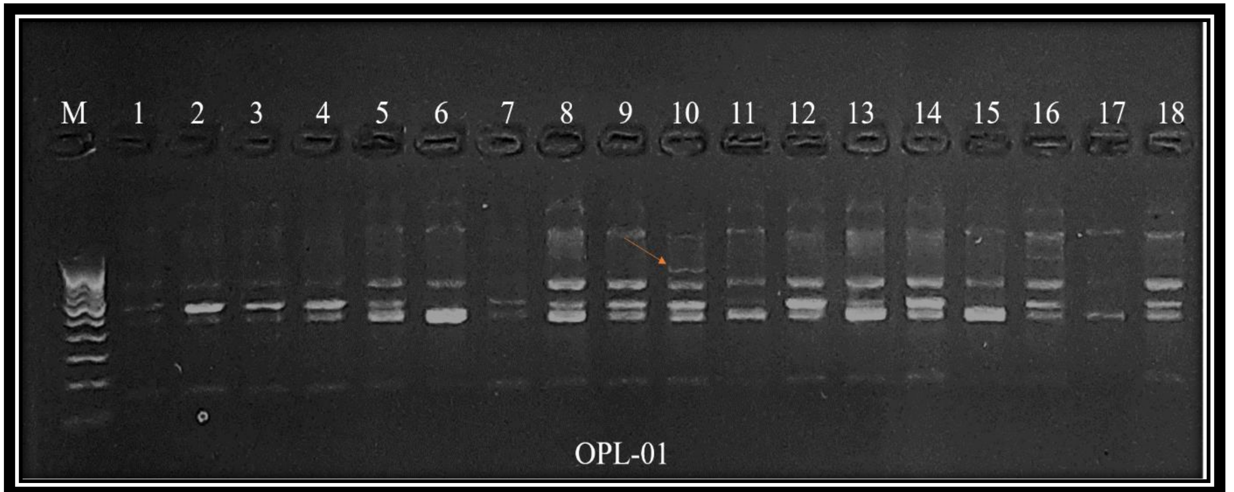
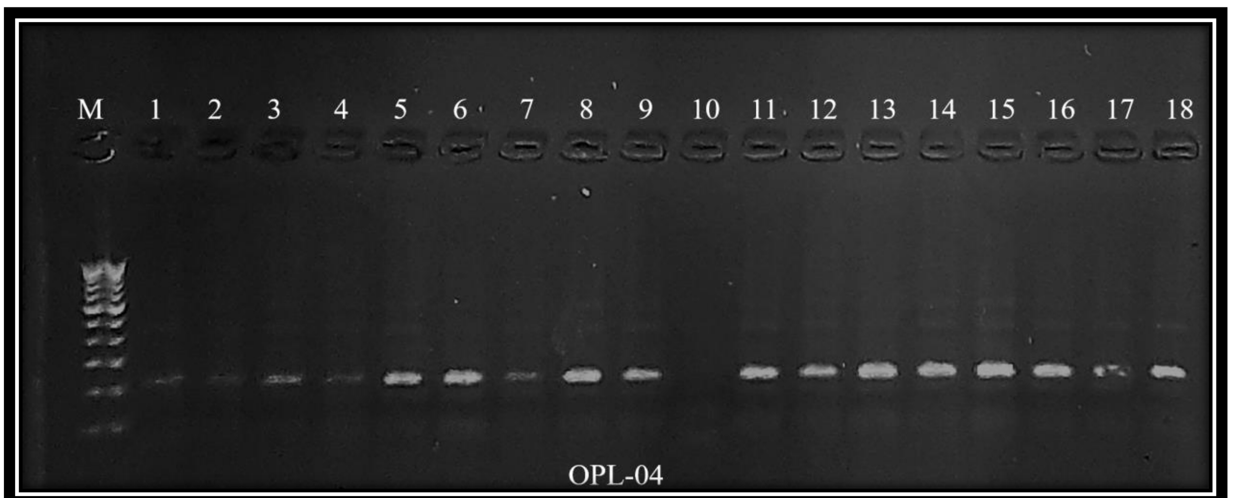


Plate 4.1: Agarose gel electrophoresis of amplified products obtained with RAPD primers OPA-16, OPC-05and OPD-05. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





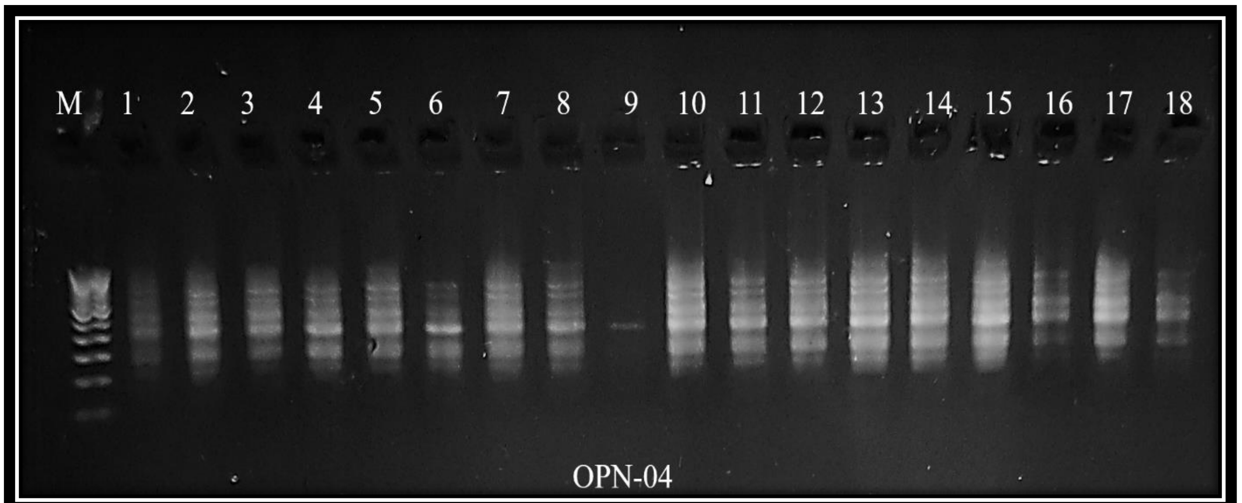
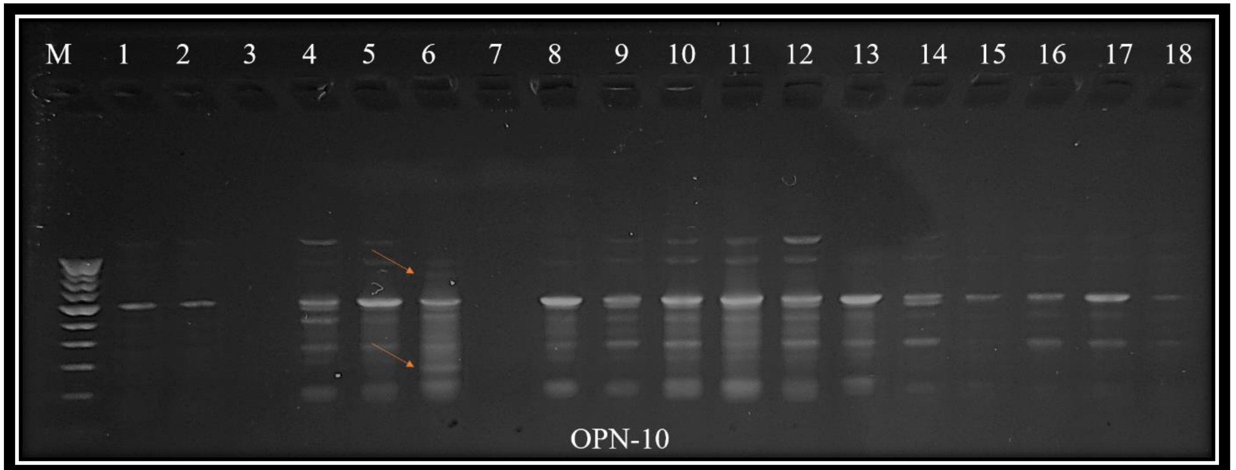


Plate 4.2: Agarose gel electrophoresis of amplified products obtained with RAPD primers OPL-01, OPL-04 and OPN-04. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)



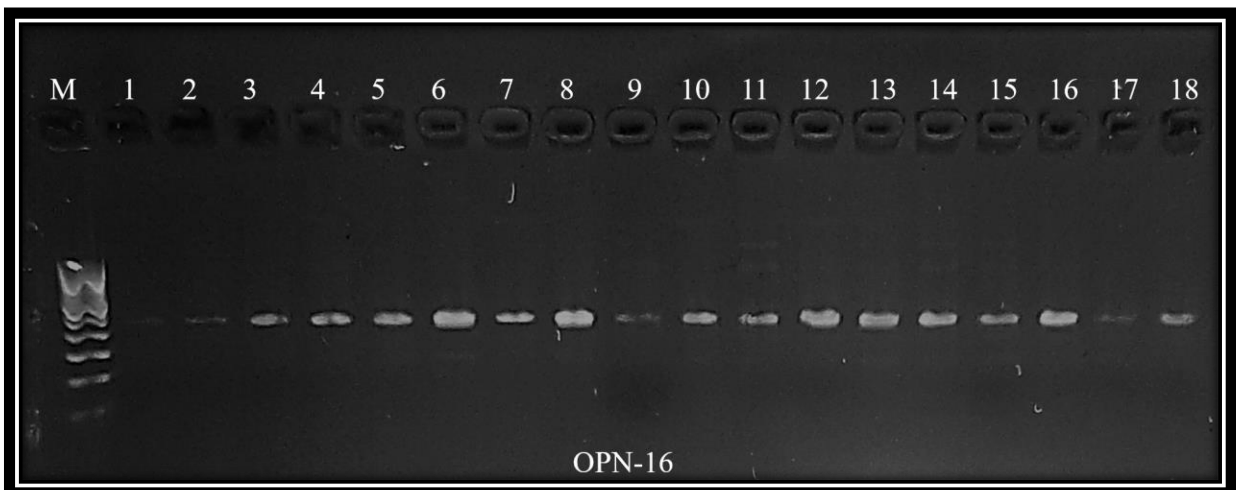
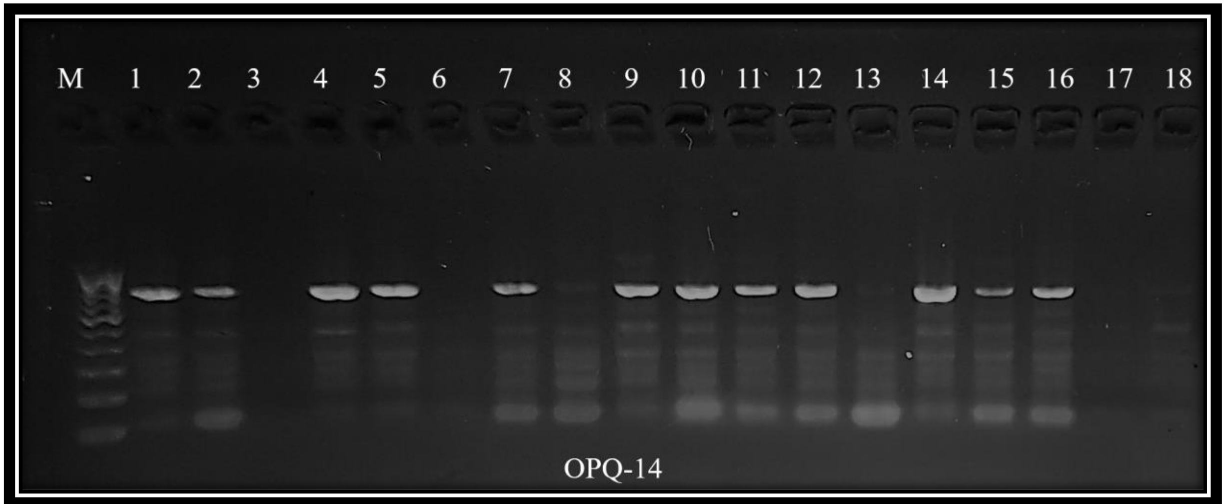
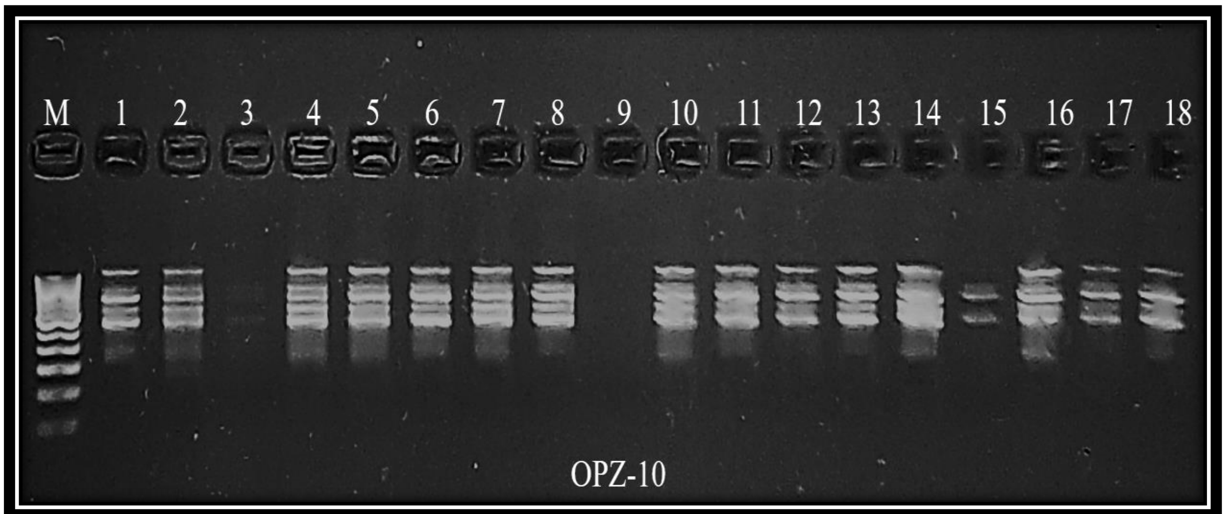


Plate 4.3: Agarose gel electrophoresis of amplified products obtained with RAPD primers OPN-10, OPN-13 and OPN-16. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





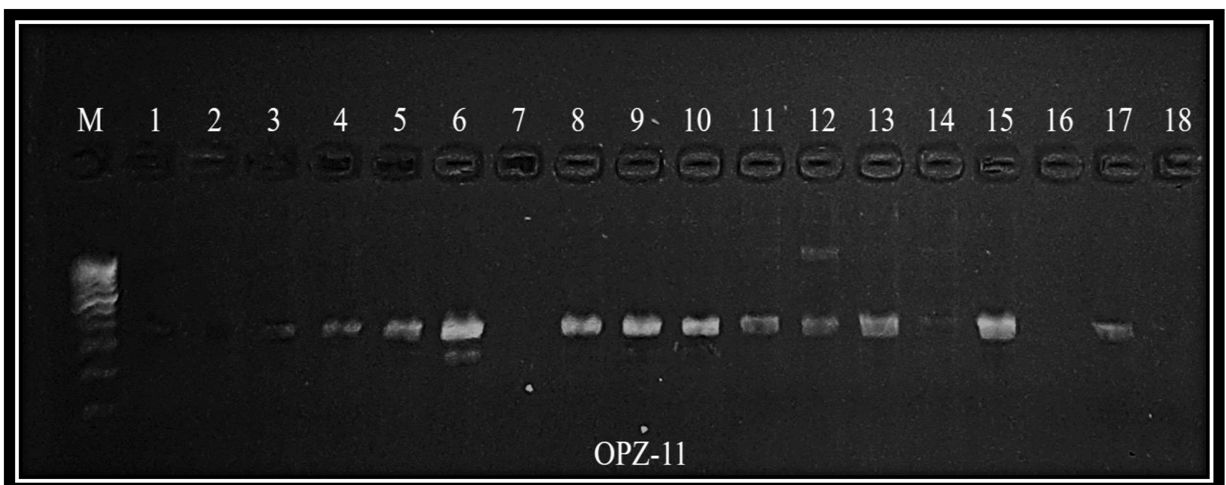
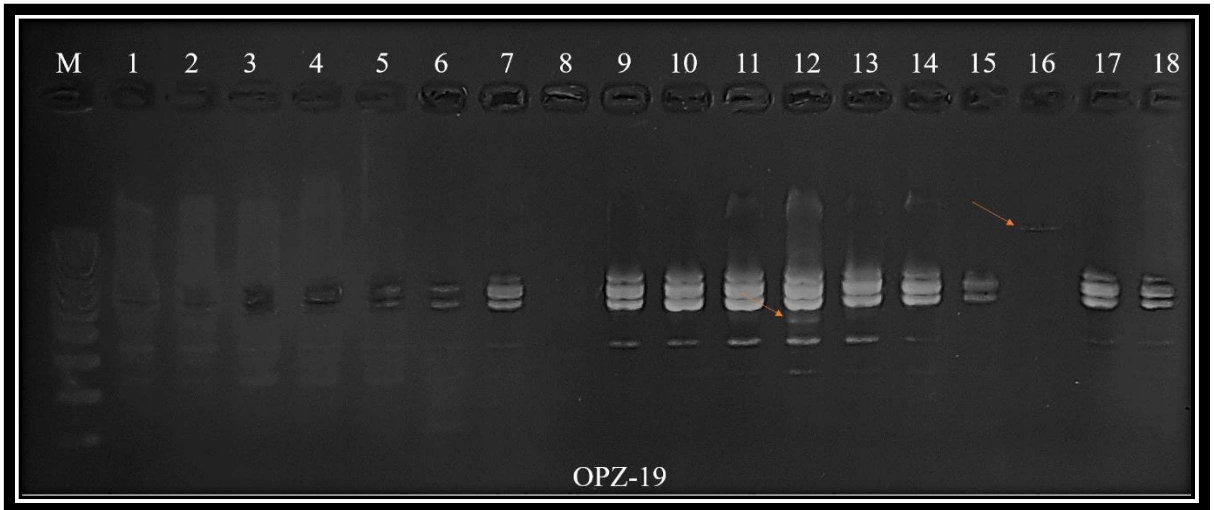
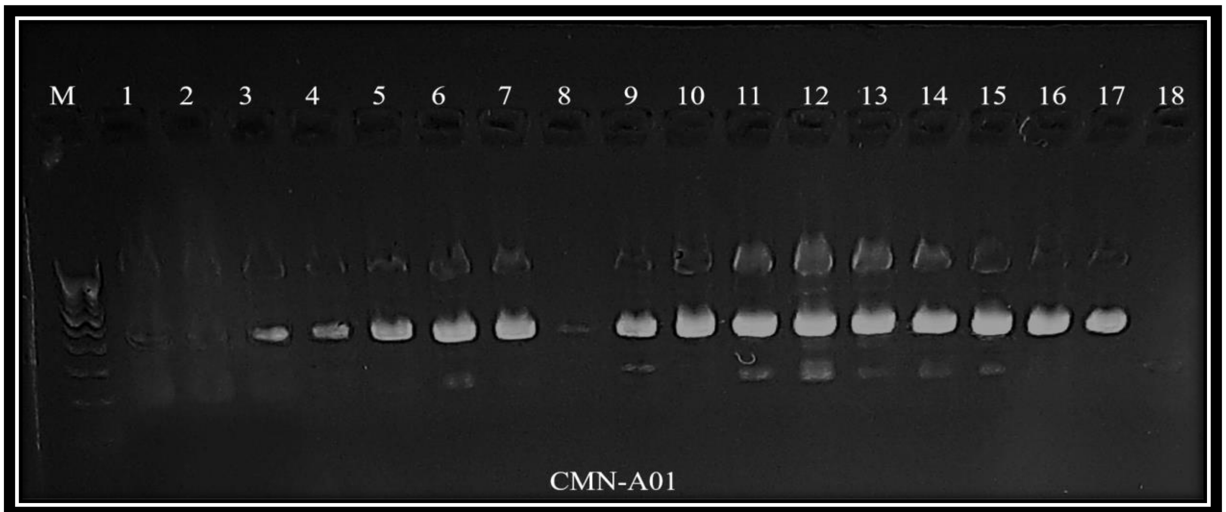


Plate 4.4: Agarose gel electrophoresis of amplified products obtained with RAPD primers OPQ-14, OPZ-10 and OPZ-11. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





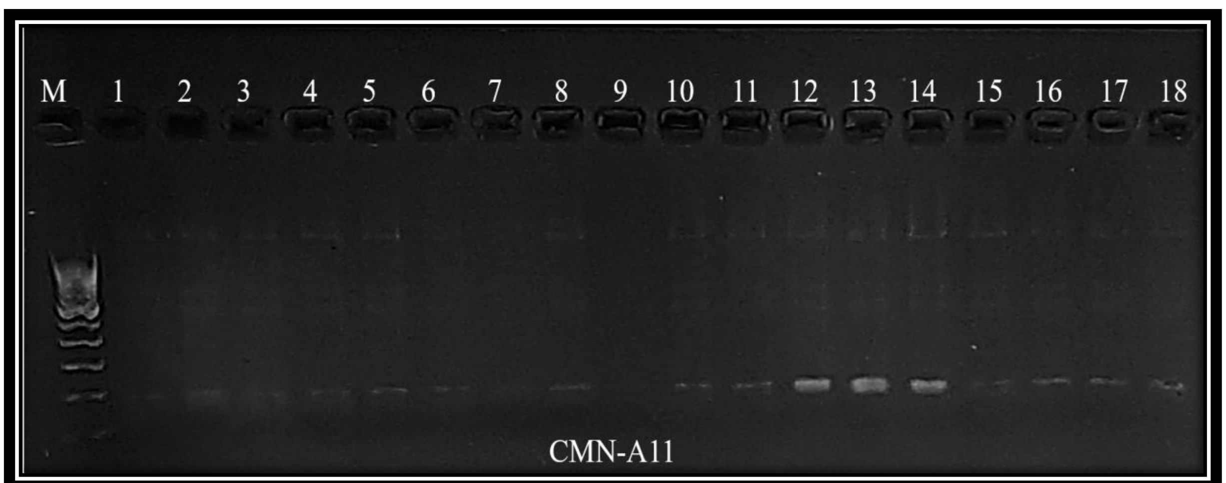
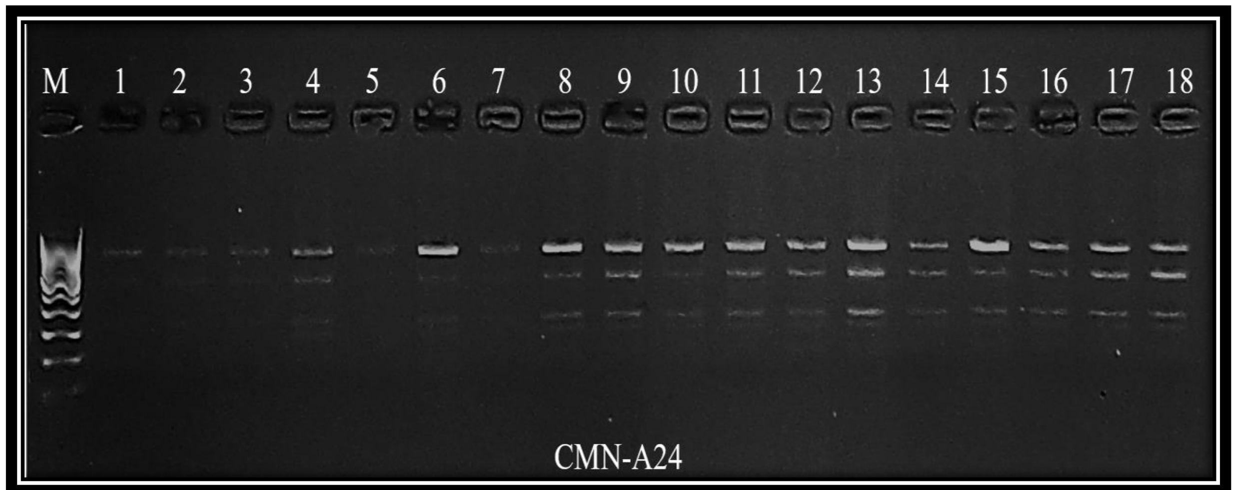
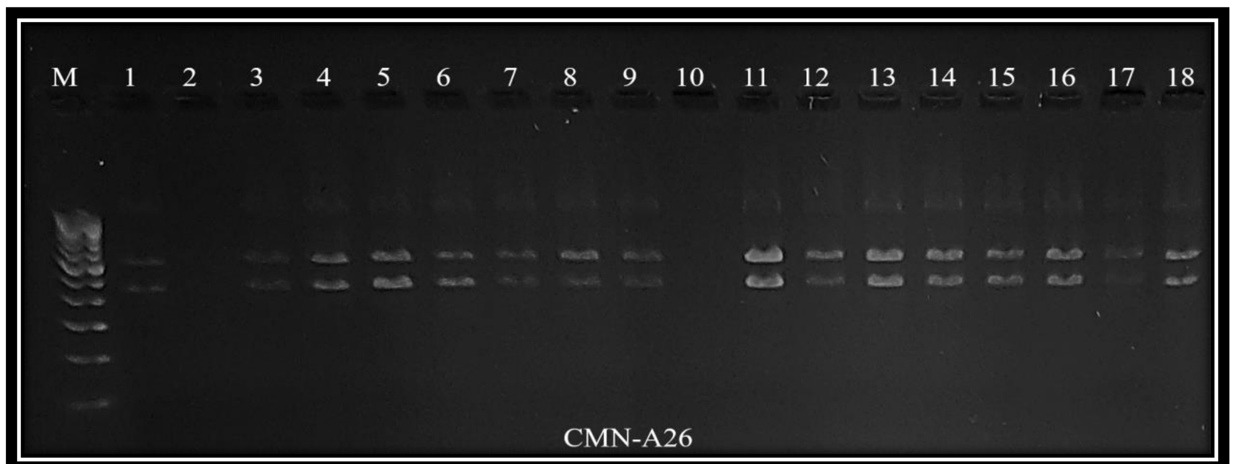


Plate 4.5: Agarose gel electrophoresis of amplified products obtained with RAPD primers OPZ-19, CMN-A01 and CMN-A11. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





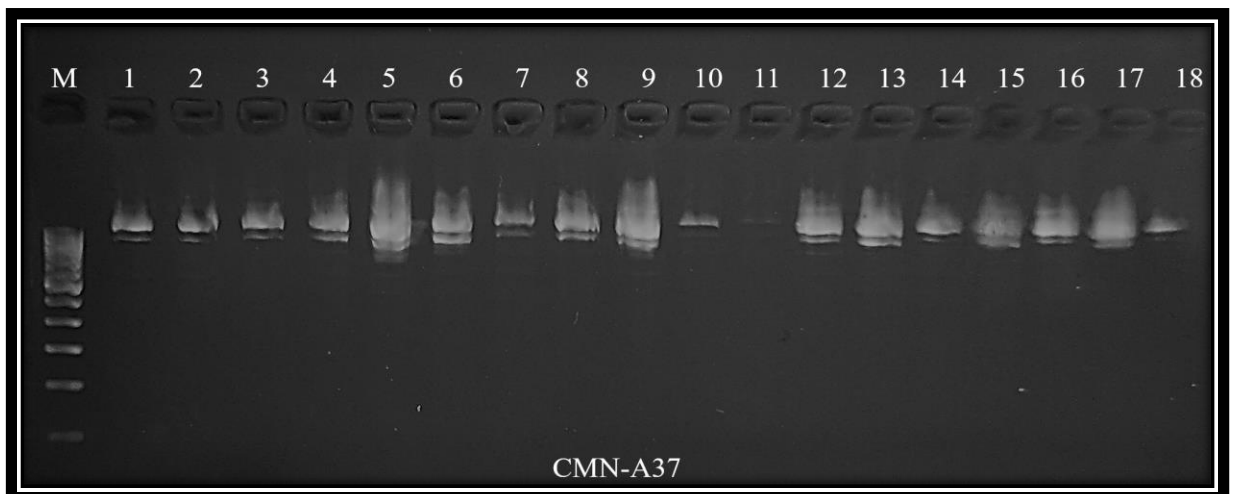
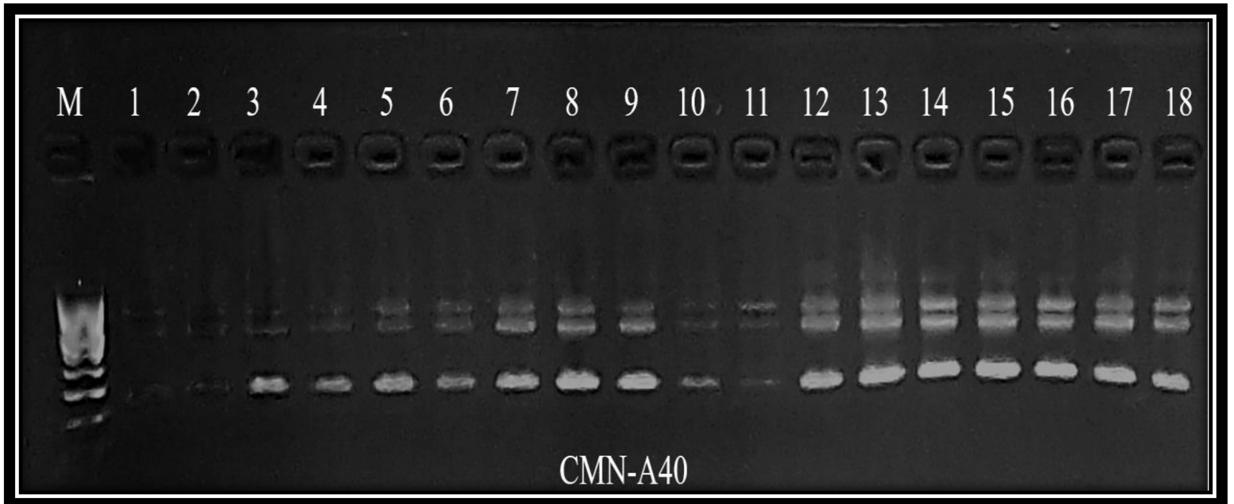
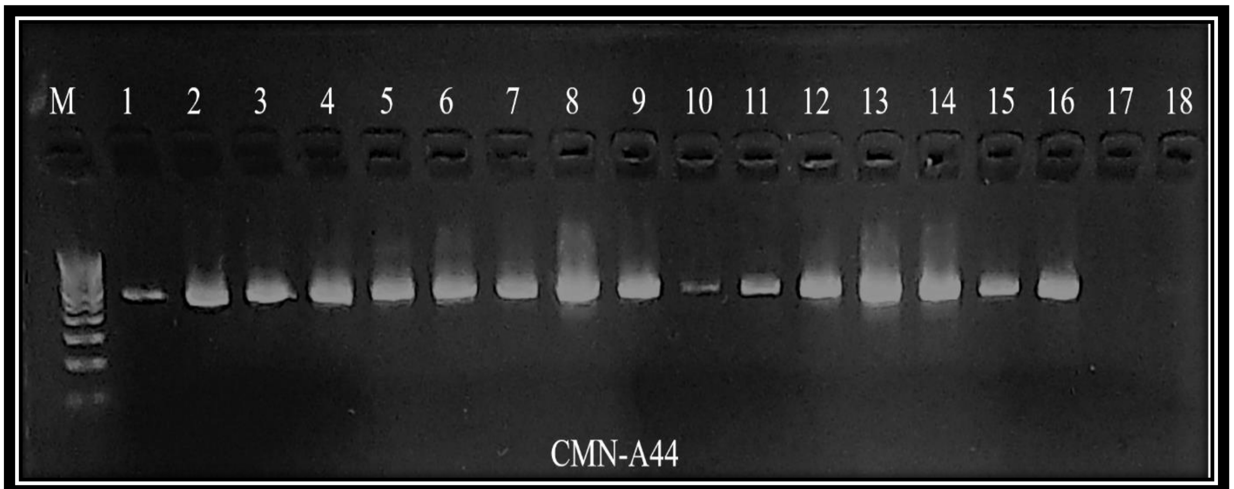


Plate 4.6: Agarose gel electrophoresis of amplified products obtained with RAPD primers CMN-A24, CMN-A26 and CMN-A37. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





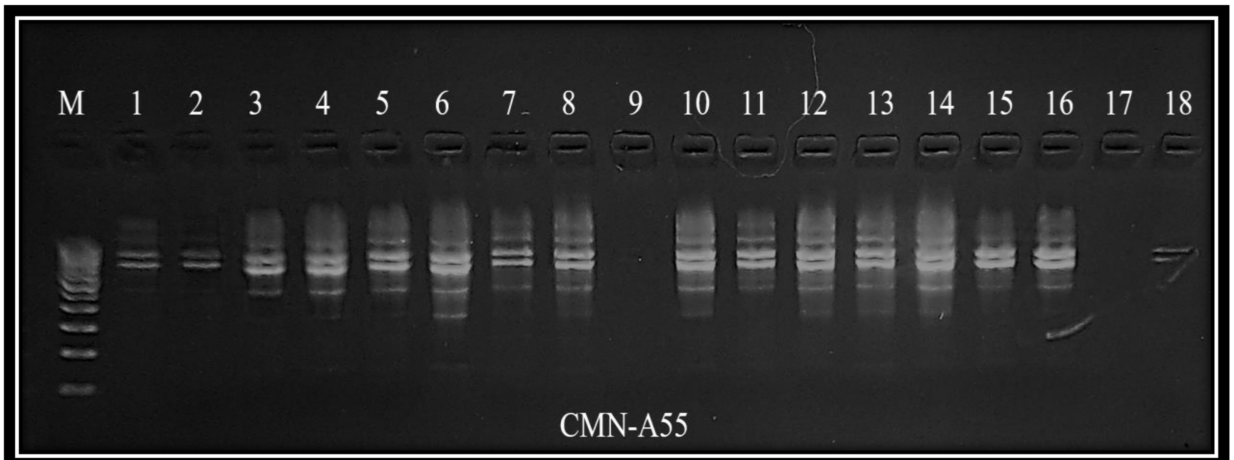
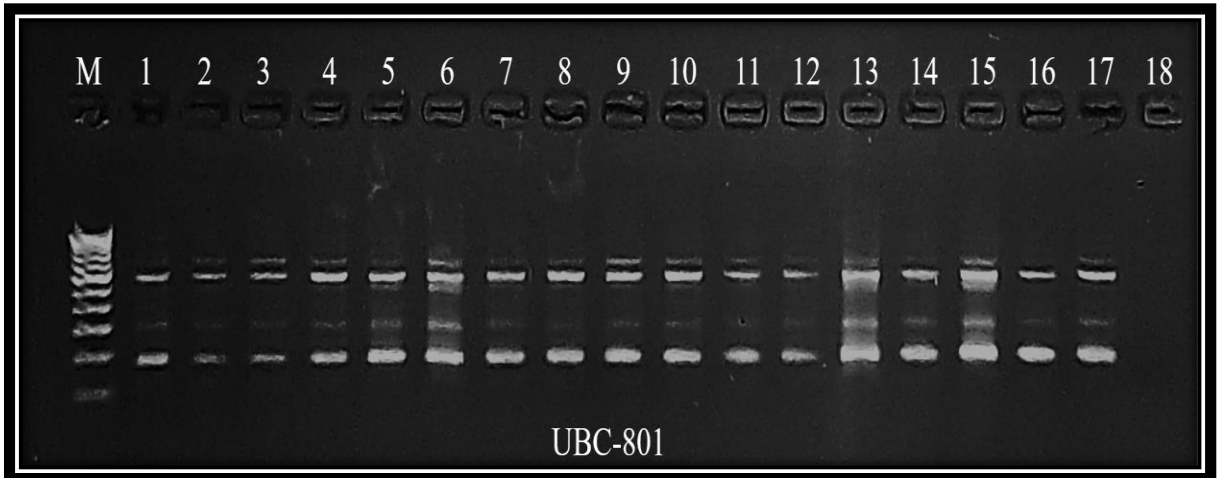
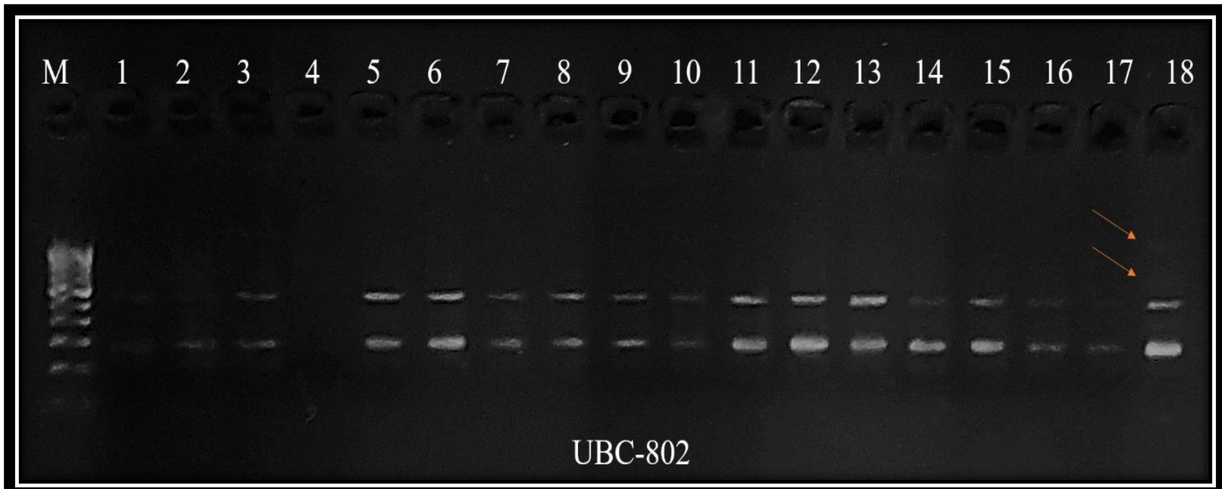


Plate 4.7: Agarose gel electrophoresis of amplified products obtained with RAPD primers CMN-A40, CMN-A44 and CMN-A55. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





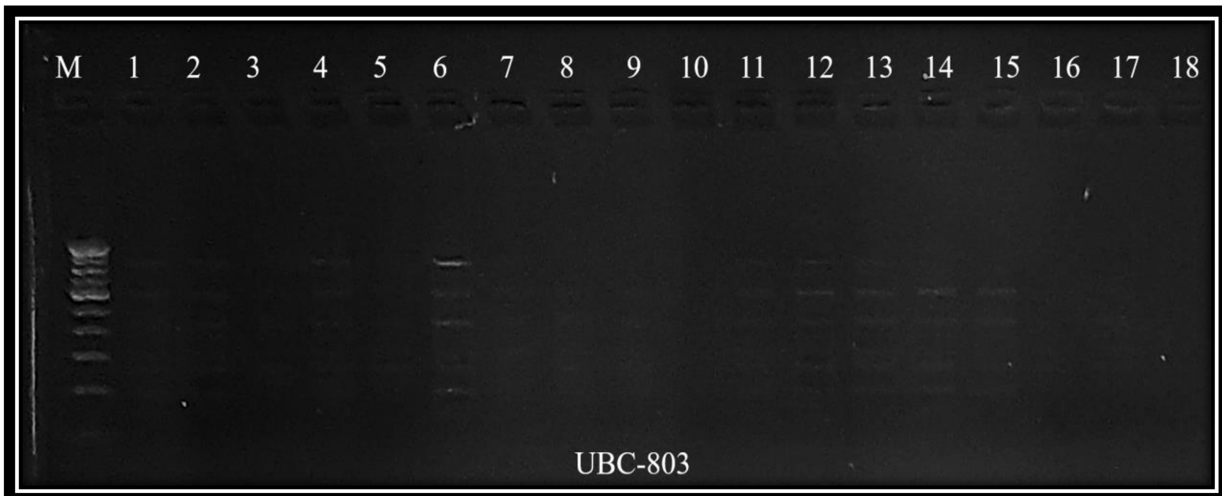
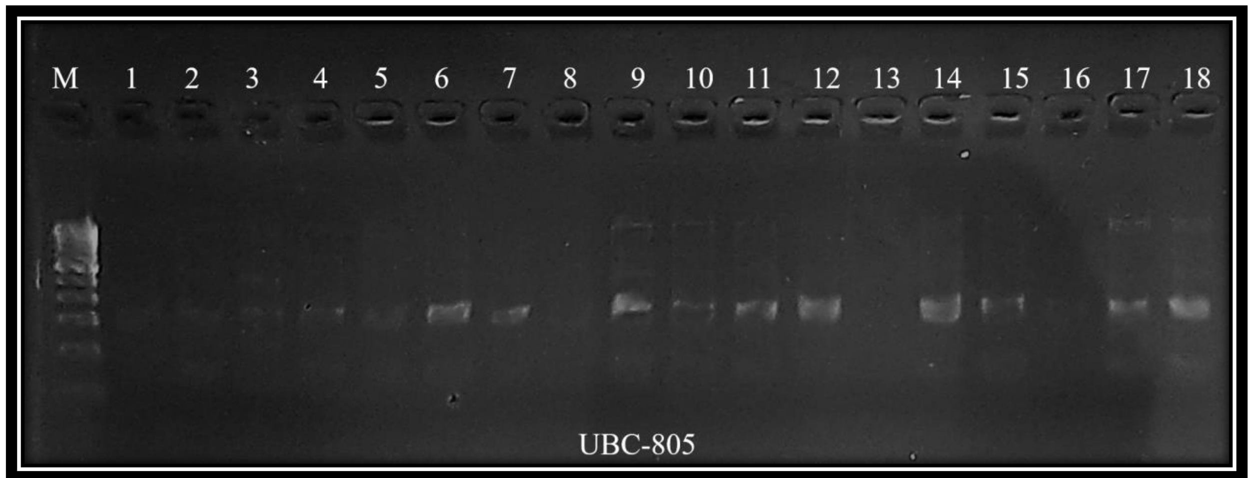
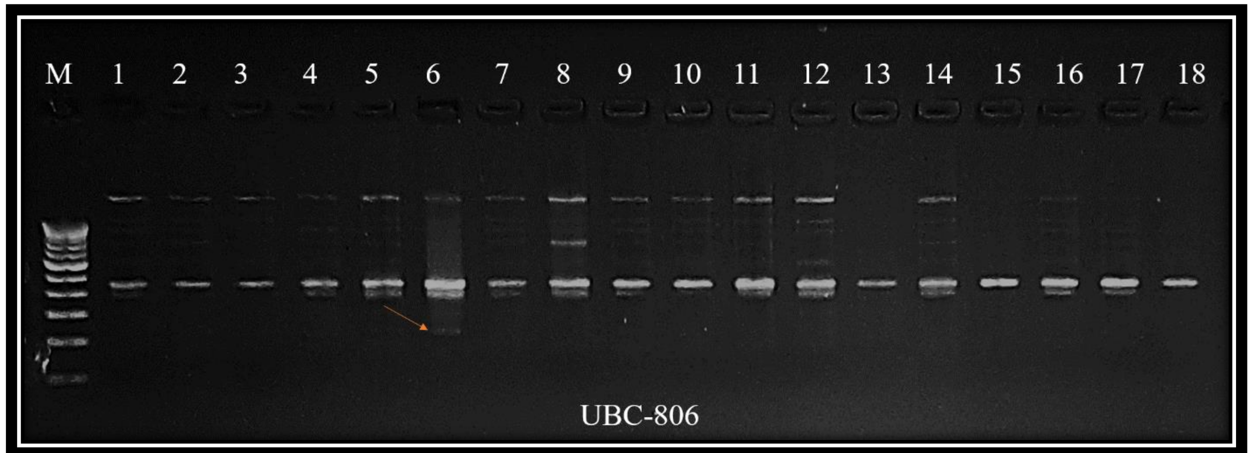


Plate 4.8: Agarose gel electrophoresis of amplified products obtained with ISSR primers UBC-801, UBC-802 and UBC-803. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





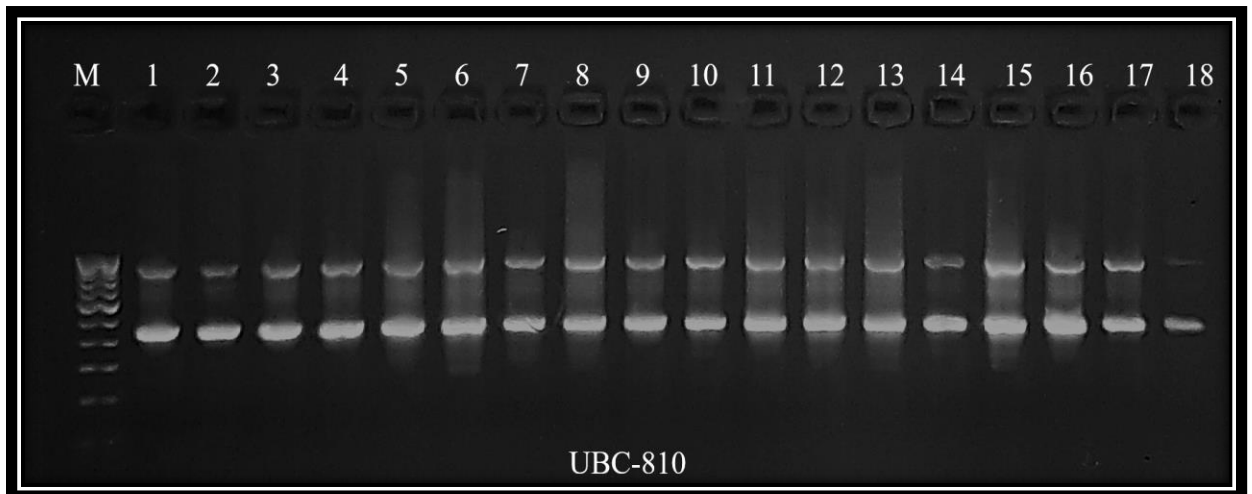
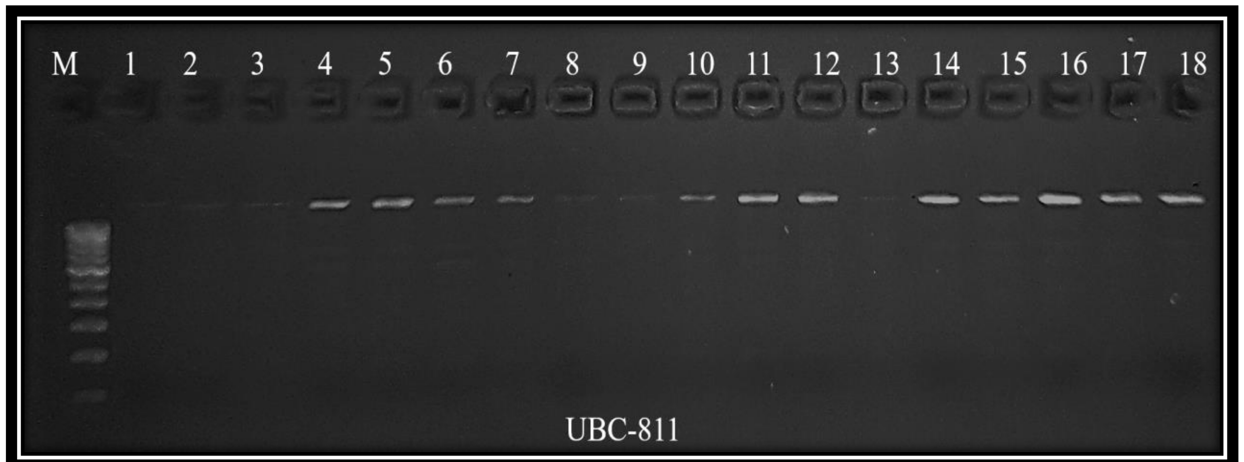
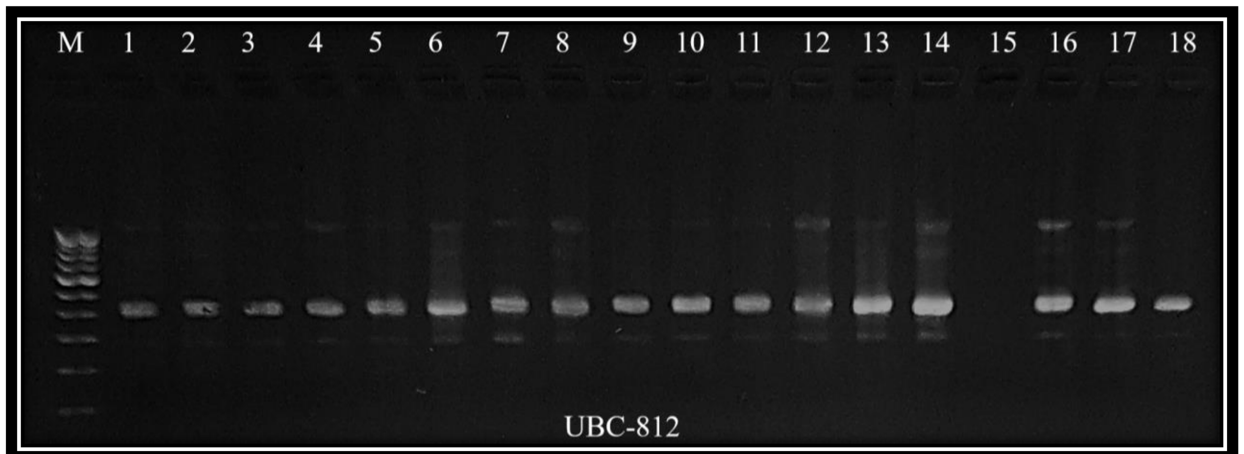


Plate 4.9: Agarose gel electrophoresis of amplified products obtained with ISSR primers UBC-805, UBC-806 and UBC-810. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





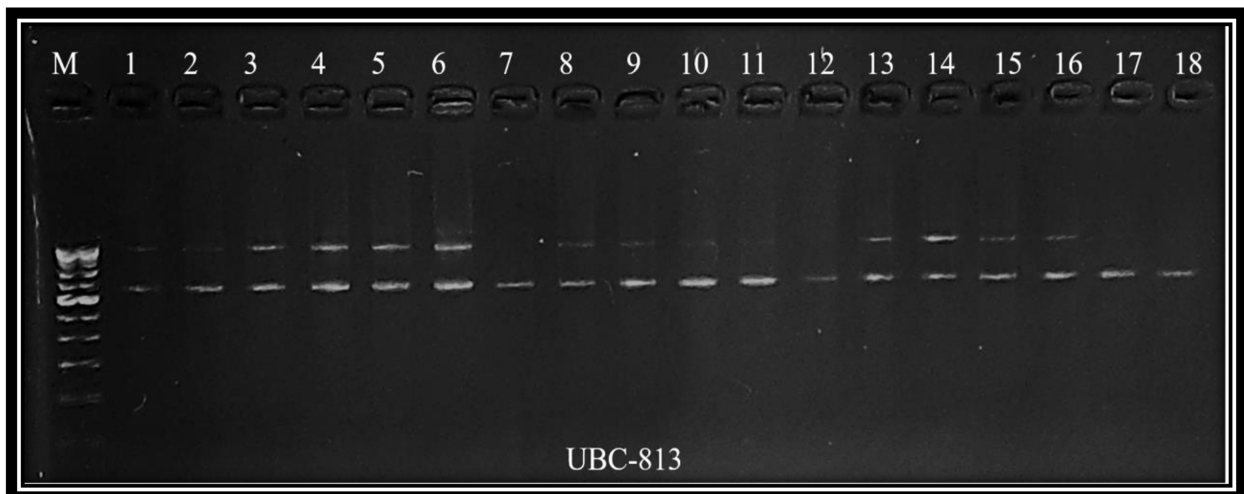
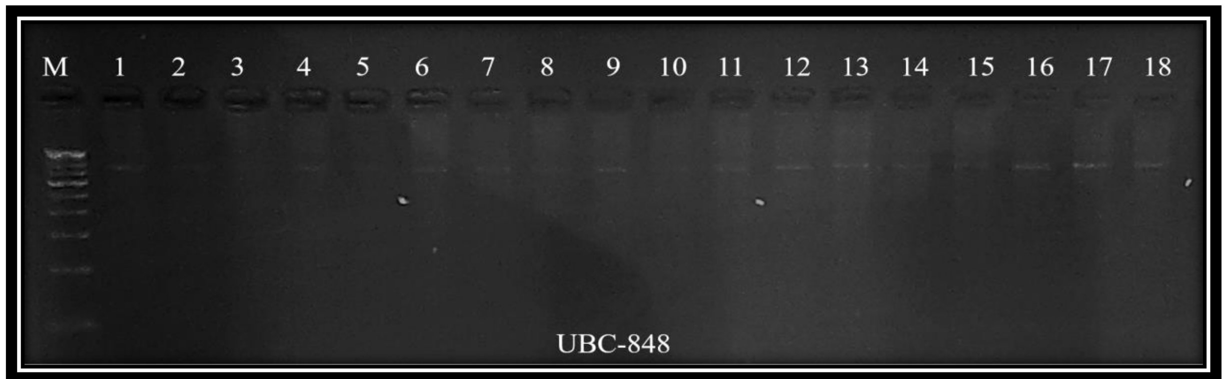
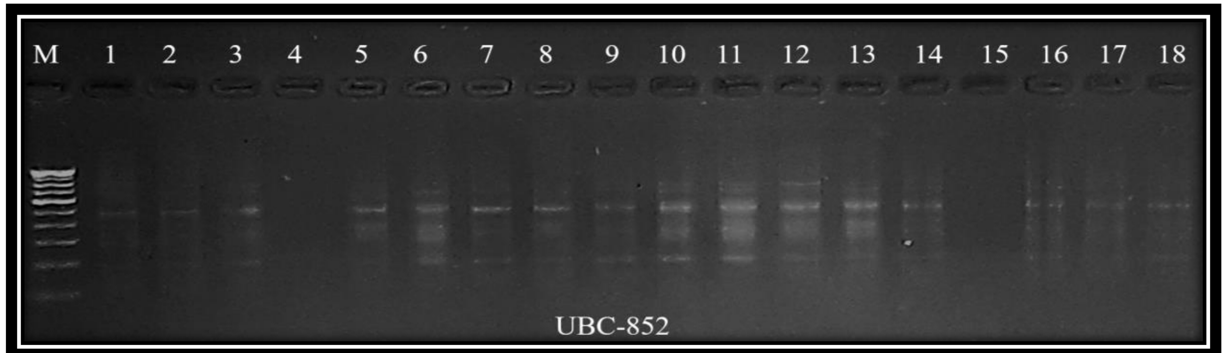
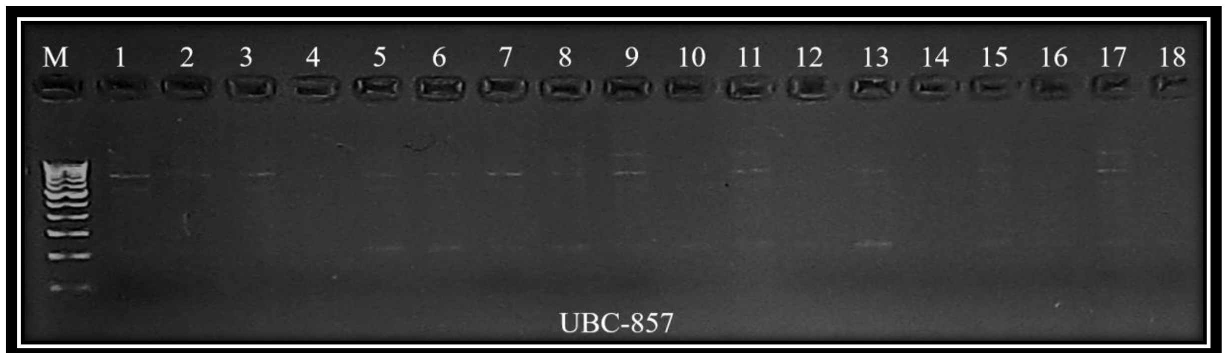


Plate 4.10: Agarose gel electrophoresis of amplified products obtained with ISSR primers UBC-811, UBC-812 and UBC-813. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)







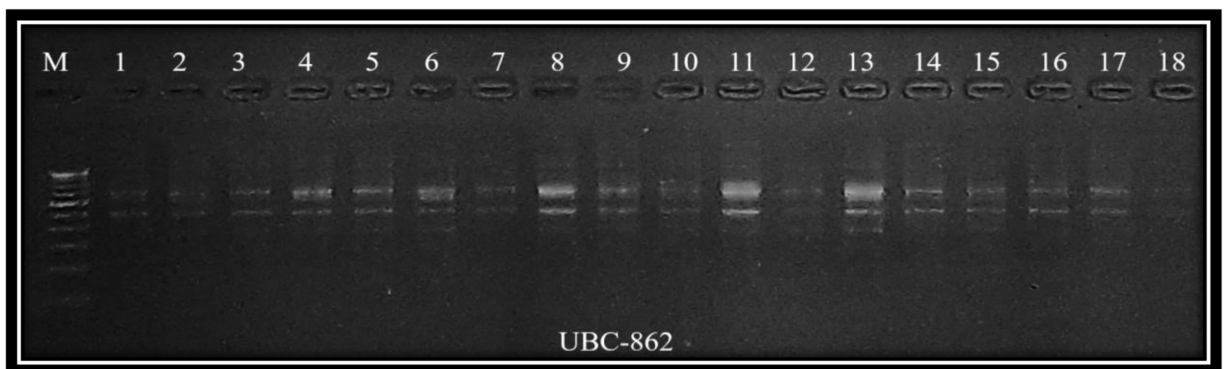
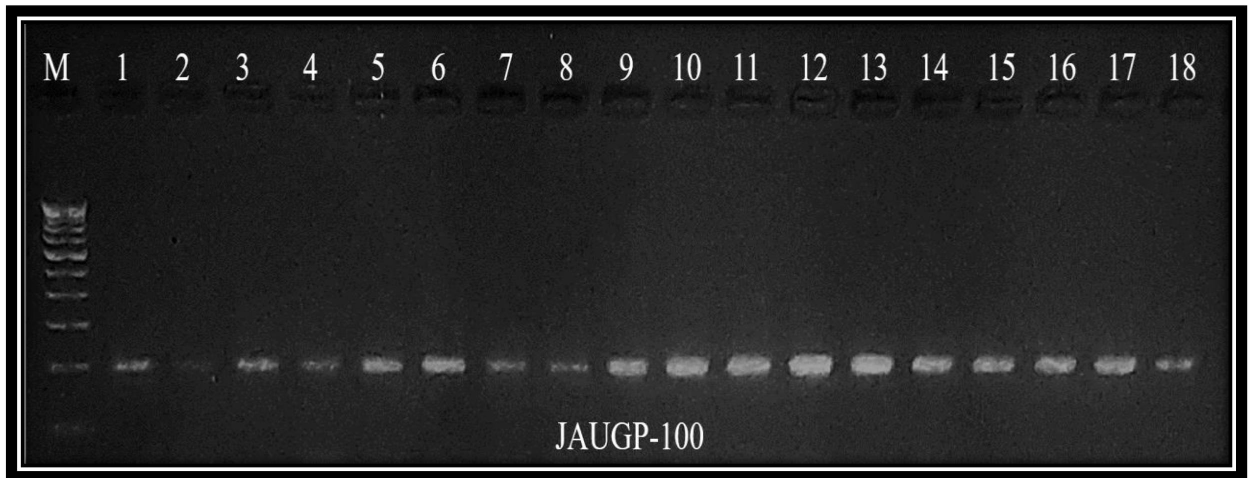
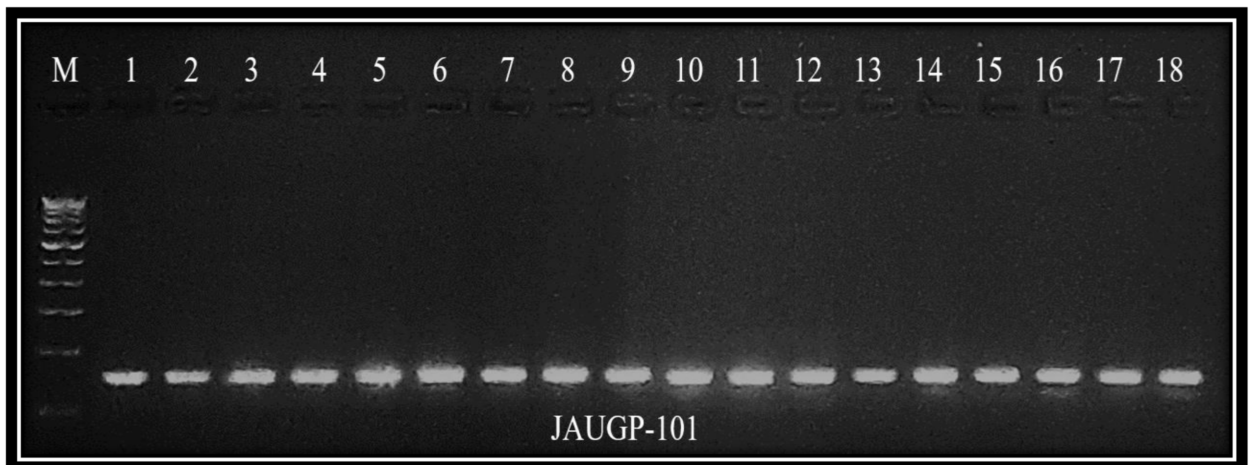


Plate 4.11: Agarose gel electrophoresis of amplified products obtained with ISSR primers UBC-848, UBC-852, UBC-857 and UBC-862. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





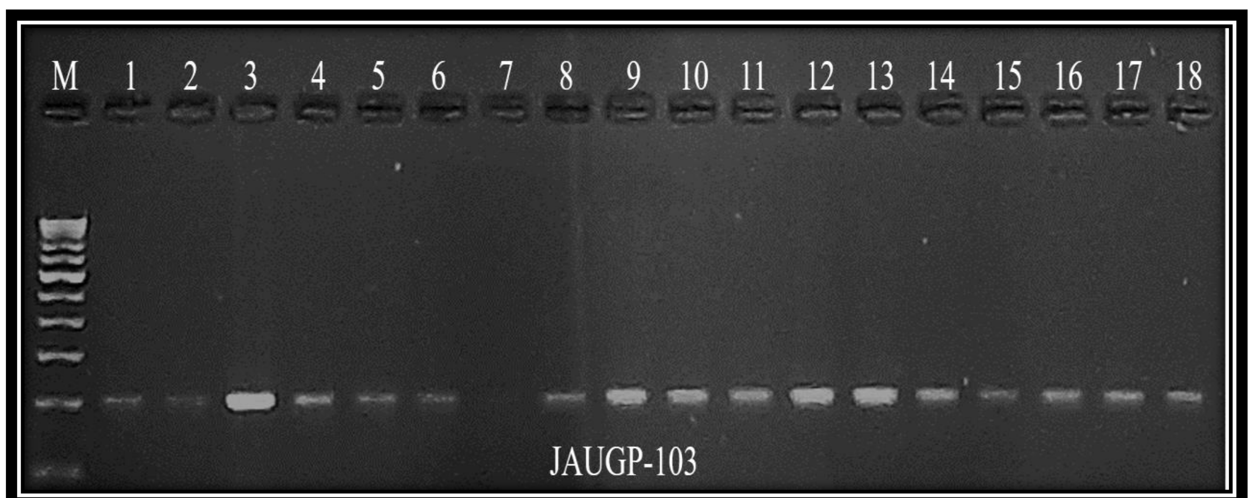
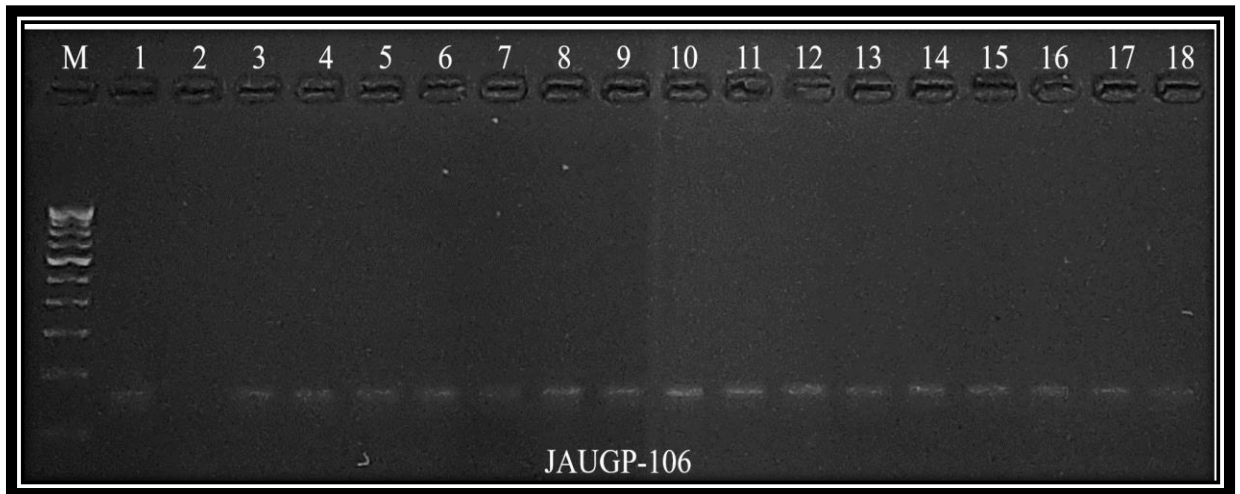
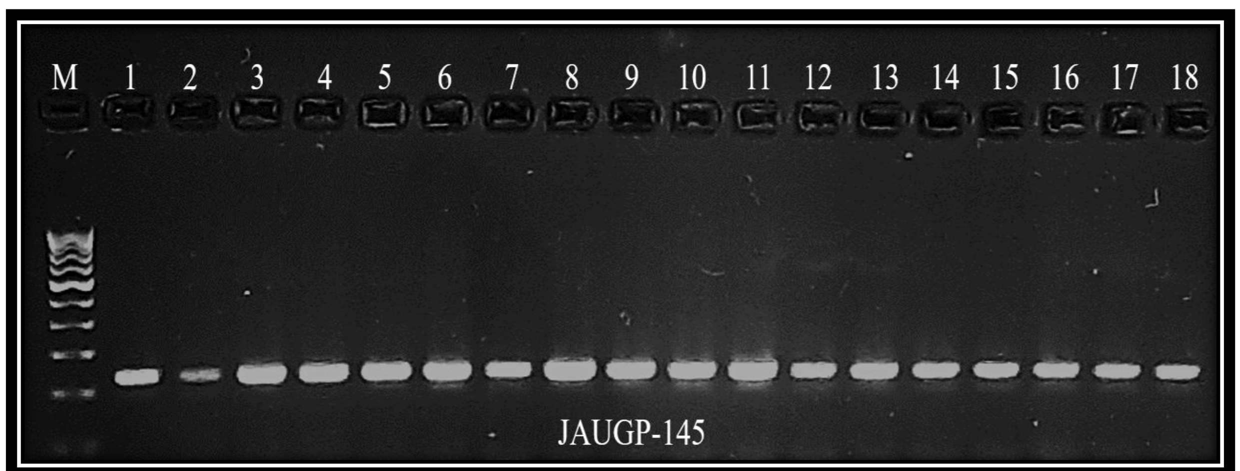


Plate 4.12: Agarose gel electrophoresis of amplified products obtained with SSR primers JAUGP-100, JAUGP-101 and JAUGP-103. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





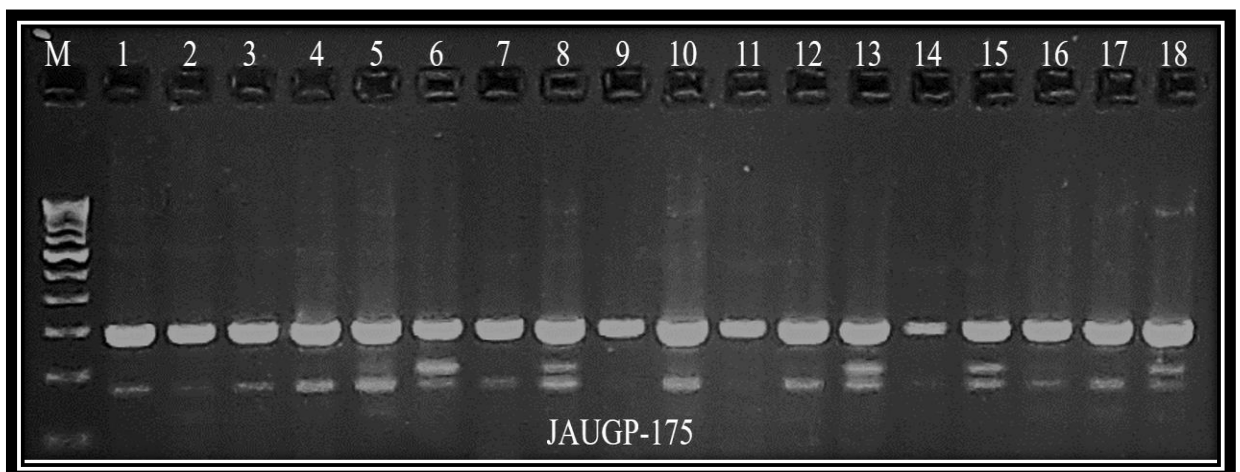


Plate 4.13: Agarose gel electrophoresis of amplified products obtained with SSR primers JAUGP-106, JAUGP-145 and JAUGP-175. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

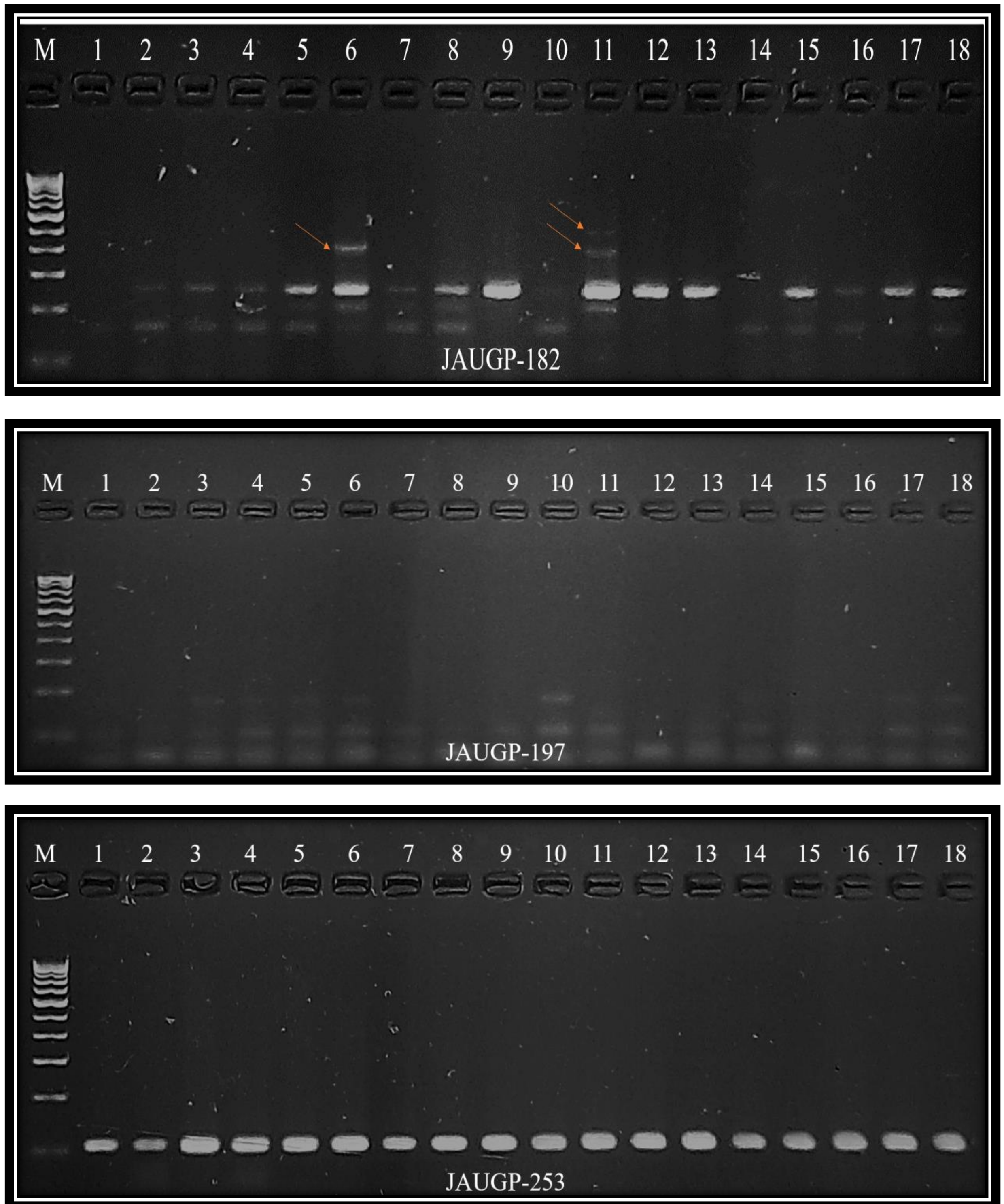
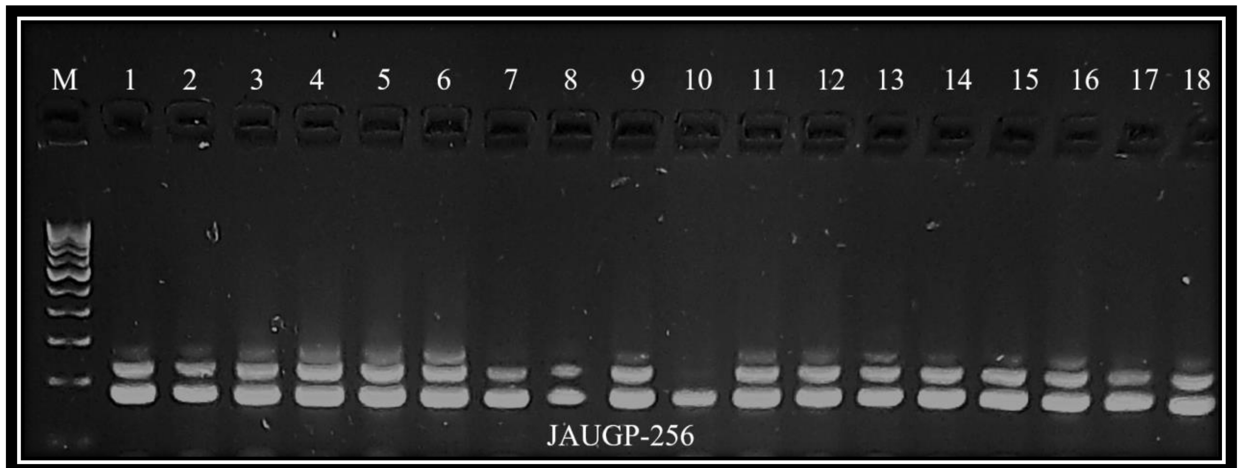
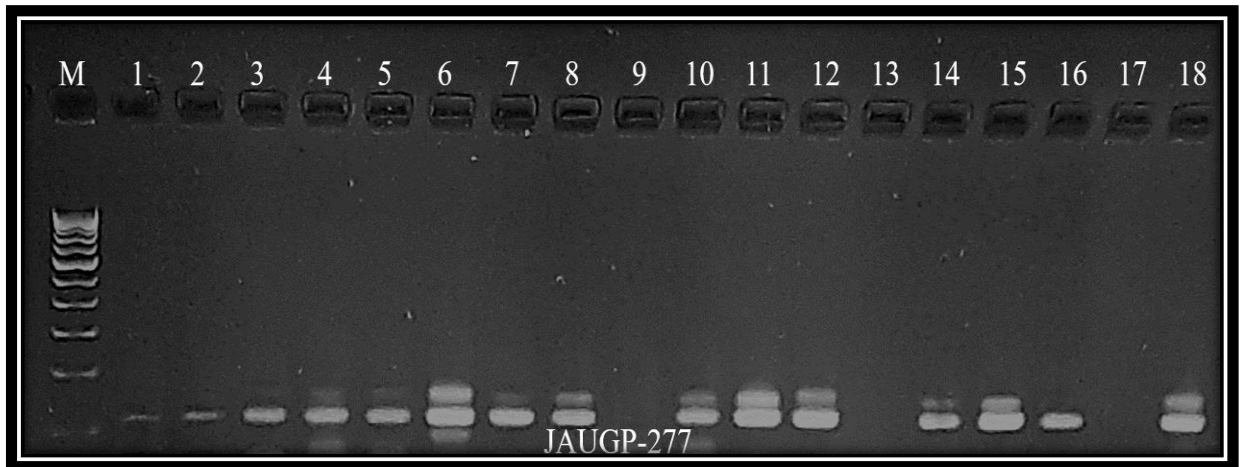


Plate 4.14: Agarose gel electrophoresis of amplified products obtained with SSR primers JAUGP-182, JAUGP-197 and JAUGP-253. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





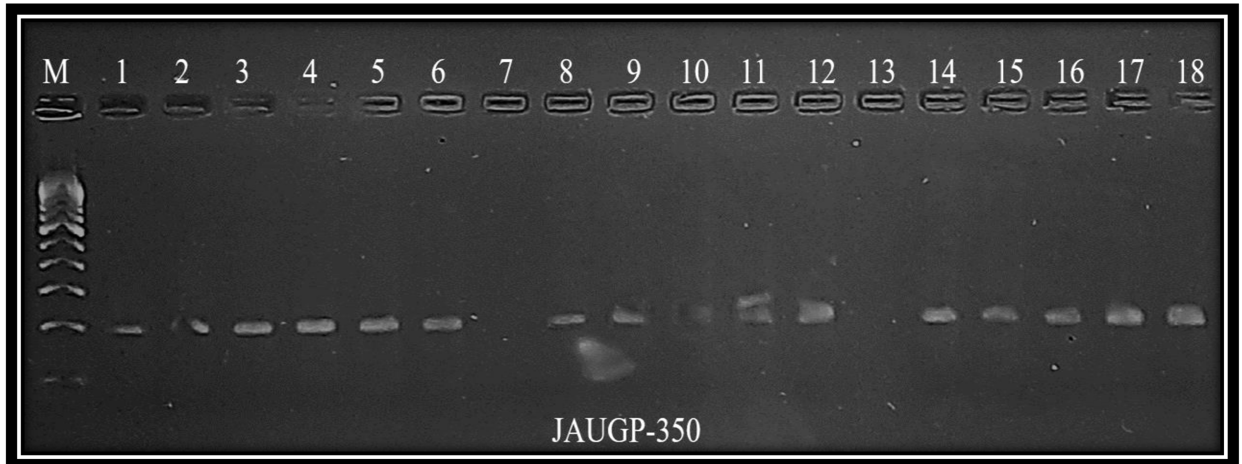
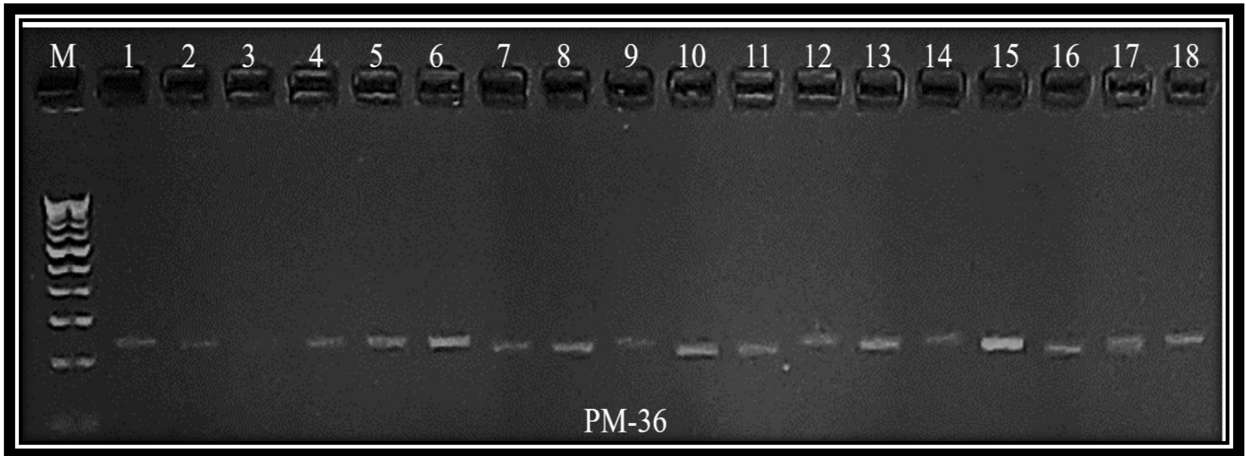
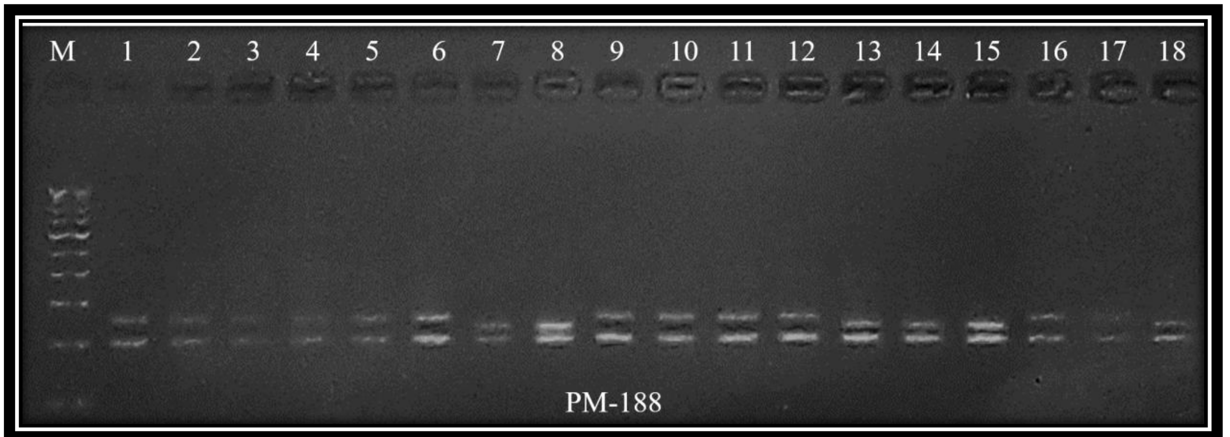


Plate 4.15: Agarose gel electrophoresis of amplified products obtained with SSR primers JAUGP-256, JAUGP-277 and JAUGP-350. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





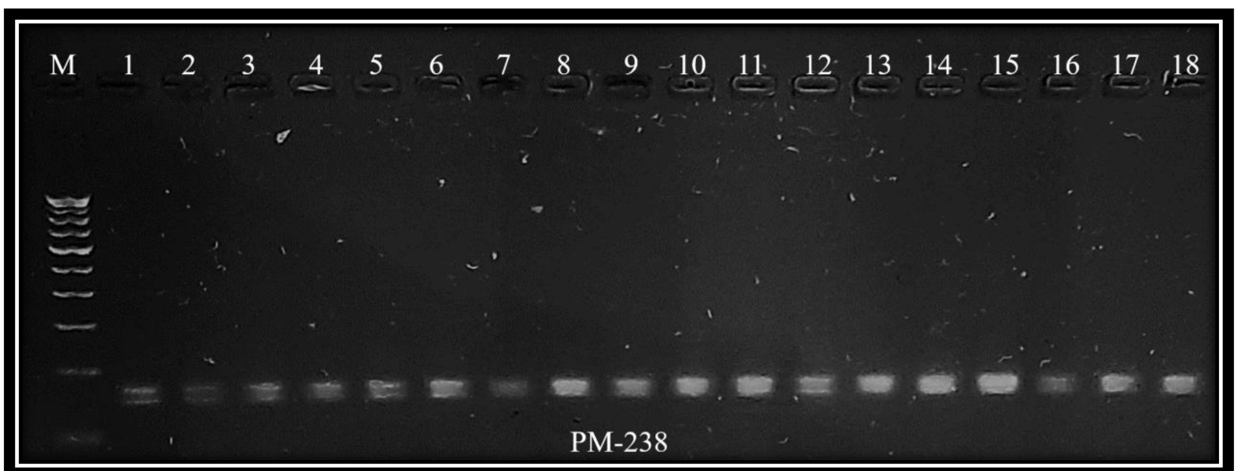
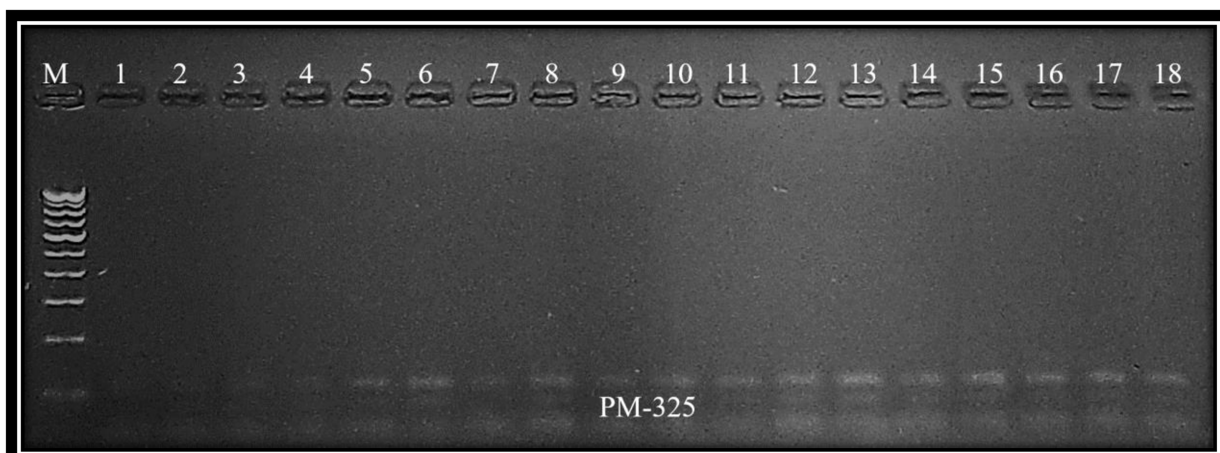
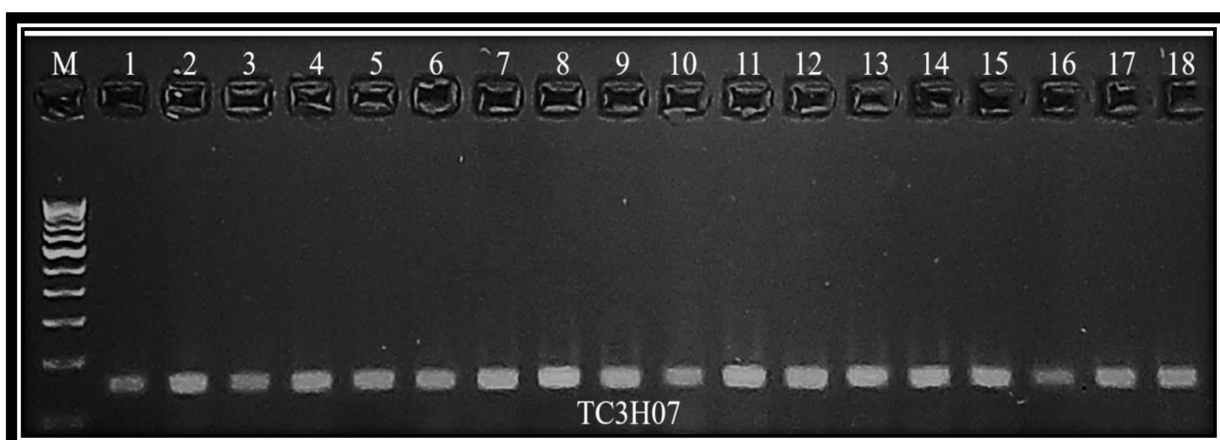


Plate 4.16: Agarose gel electrophoresis of amplified products obtained with SSR primers PM-36, PM-188 and PM-238. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)





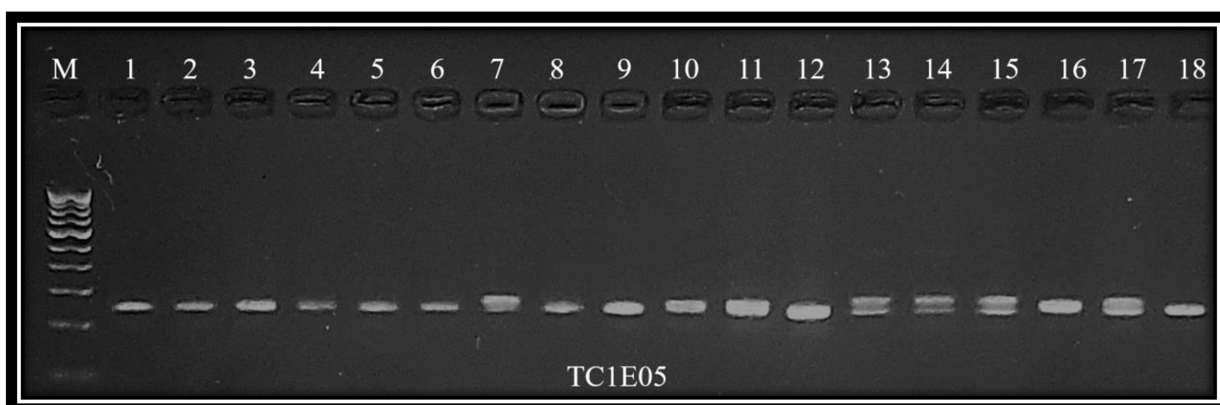


Plate 4.17: Agarose gel electrophoresis of amplified products obtained with SSR primers PM-325, TC3H07 and TC3HO9. (M: DNA ladder; 1: GAUG-10; 2: GG-11; 3: GG-16; 4: GJG-17; 5: GG-41; 6: GJG-HPS-1; 7: GG-21; 8: GJG-22; 9: GG-HPS-2; 10: KDG-123; 11: KDG-128: 12: Kaushal; 13: GG-6; 14: GG-7; 15: GJG-9; 16: GJG-32; 17: JL-501; 18: TG-37A)

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