"CHARACTERIZATION OF OKRA (Abelmoschus esculentus L. Moench) GENOTYPES THROUGH BIOCHEMICAL AND MOLECULAR MARKERS"

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IN

PLANT MOLECULAR BIOLOGY & BIOTECHNOLOGY

BY

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B.Sc. (Biotechnology)

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ANAND-388 110
2016

Registration No. 04-2098-2013

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ABSTRACT

Okra (*Abelmoschus esculentus* L. Moench) is known in many english-speaking countries as lady's fingers is a flowering plant in the *Malvaceae* family. Okra is cultivated for its fibrous fruits or pods containing round seeds. The fruits are harvested when immature, and it can be cooked in a variety of ways.

Morphological attributes like plant height, fruit length, fruit girth, fruit volume and fruit density were measured, as well as various phytochemicals from seed *viz*., flavanoids, glycosides, saponins, tannins, alkaloids were studied and it resulted flavanoids, glycosides, saponins are present but tannins and alkaloids are absent.

Different biochemical attribute like moisture, true protein, crude protein, total lipids and free fatty acids analyzed from okra seeds.

Isozyme electrophoresis of polyphenol oxidase (PPO), peroxidase (POX), and esterase were found useful for identification of cultivars as well as may serve as marker isozyme for okra genotypes. In PPO (Polyphenol oxidase) maximum two numbers of bands were observed on the basis of intensity at 0.34 and 0.56 Rm value. In polyphenol oxidase maximum two numbers of bands were observed on the basis of intensity at 0.29 and 0.58 Rm value, while in esterase exhibited maximum six number of bands were found on the basis of intensity at 0.25, 0.27, 0.53, 0.56, 0.60 and 0.65 Rm value.

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of seed proteins showed variability or genetic diversity of okra cultivars. In present investigation total number of fifteen bands was observed among them red okra is distinguished the diverse genotype than all among thirty genotypes.

The molecular characterization of okra genotypes for genetic diversity was done by using polymerase chain reaction (PCR) based molecular markers *viz.,* random amplified polymorphic DNA (RAPD) and Simple sequence repeat (SSR). The RAPD analysis with 8 primers produced 72 total loci and total bands produced were 1074. Out of these 55 were polymorphic and hence the total polymorphism percentage was found to be 74.60%. In the SSR analysis 15 markers were amplified and produced 72 alleles. The average number of alleles per locus was found to be 4.80, while effective number of alleles was 1.46. The maximum number of alleles was 8 which were recorded

for markers OK-8, OK-1, OK-6, OK-11, OK-12 and OK-14 were produced three alleles.

Genetic variability of thirty genotypes studied through molecular markers resulted that Red okra is most diverse genotype as compared to other okra genotypes; it is also diversified by various morphological characters.

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CERTIFICATE

This is to certify that the thesis entitled **"CHARACTERIZATION OF OKRA (*Abelmoschus esculentus* L. Moench) GENOTYPES THROUGH BIOCHEMICAL AND MOLECULAR MARKERS"** submitted by **KINJAL HARIBHAI BHUTAKA (Reg. No. 04-2098-2013)** in partial fulfillment of the requirements for the award of the degree of **Master of Science** in **Plant Molecular Biology and Biotechnology** of the Anand Agricultural University is a record of bonafide research work carried out by her under my personal guidance and supervision. The thesis has not previously formed the basis for the award of any degree, diploma or other similar title.

Place: Anand **(J. J. Dhruve)**
Date: /07/2016 Major Advisor

DECLARATION

This is to declare that the whole of the research work reported here in the thesis for the partial fulfillment of the requirements for the degree of **Master of Science** in **Plant Molecular Biology and Biotechnology**, by the undersigned is the results of investigation done by her under the direct guidance and supervision of **Dr. J. J. Dhruve,** Associate Professor, Department of Biochemistry, B. A. College of Agriculture, Anand Agricultural University, Anand and no part of the work has been submitted for any other degree so far.

Place: Anand

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Place: Anand

Date: /07/ 2016 **(Kinjal H. Bhutaka)**

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ABBREVIATIONS

1)	%	Percentage
2)	µl	Micro litre
3)	^{0}C	Degree Celsius
4)	BC	Before Christ
5)	bp	Base pair
6)	C.D.	Critical difference
7)	C.V.	Coefficient of variation
8)	COPH	Cophenetic Correlation
9)	CTAB	Cetyl Trimethyl Ammonium Bromide
10)	DNA	Deoxyribonucleic Acid
11)	DPPH	1, 1-diphenyl-2-picryl hydrazyl radical
12)	e.g.	Example
13)	EDTA	Ethylene Diamine Tetra Acetic Acid
14)	ET	Ethylene
15)	EtBr	Ethidium Bromide
16)	etc	Etcetera
17)	FAO	Food and Agricultural Organization of United Nation
18)	gm	Gram
19)	GS	Genetic Similarity
20)	ha	Hectare
21)	HCl	Hydrogen Chloride
22)	hrs	Hours
23)	HSP	Heat shock protein
24)	Kb	Kilo base

25)	Kg	Kilo gram
26)	M	Molar
27)	mA	MilliAmpere
28)	mg	Milligram
29)	$MgCl_2$	Magnesium Chloride
30)	min	Minutes
31)	ml	Mili litre
32)	Mm	Mili meter
33)	mM	Milli Molar
34)	Mol. wt.	Molecular weight
35)	N	Normal
36)	NaCl	Sodium Chloride
37)	ng	Nanogram
38)	ng/l	Nanogram per litre
39)	nm	Nano meter
40)	No.	Number
41)	OD	Optical Density
42)	PCR	Polymerase Chain Reaction
43)	Pg	Picogram
44)	pH	Negative logarithm of Hydrogen ion concentration
45)	PIC	Polymorphism Information Content
46)	POX	Peroxidase
47)	PPO	Polyphenol oxidase
48)	PVP	Polyvinylpyrolidone
49)	RAPD	Randomly Amplified Polymorphic DNA
50)	S.E.m	Standard error of mean

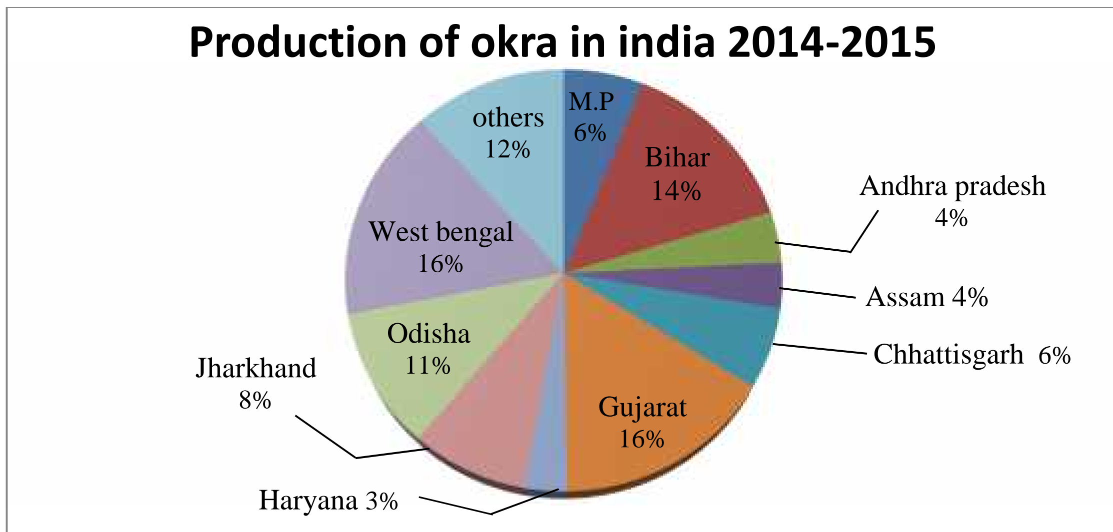
51)	Sr.	Serial
52)	SSR	Simple sequence repeat
53)	T	Temperature
54)	t	Tone
55)	TBE	Tris-Borate EDTA
56)	TE	Tris- EDTA
57)	TEMED	Tetra methyl ethylene diamine
58)	U	Units of enzyme
59)	UPGMA	Unweighted Paired Group Mathematical
60)	*Viz*.	Namely
61)	w/v	Weight per Volume
62)	µg	Microgram

CHAPTER – I

INTRODUCTION

Okra (*Abelmoschus esculentus* L. Moench), known in many English-speaking countries as ladies' fingers and bhindi is a flowering plant belongs to *Malvaceae* family. It is an economically important vegetable crop grown in tropical and sub-tropical region of the world. This crop is suitable for cultivation as a garden crop as well as on large commercial farms. It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malaysia, Brazil, Ghana, Ethiopia, Cyprus and the Southern United States. India is the major producer and exporter of okra. This crop is mainly grown in arid as well as semi-arid region of India. In India, it is grown in 531 ha. area with production of 6350 mt and productivity is 11.95 mt/ha (horticulture statistics, 2014). In India the major okra growing states are Andhra pradesh, Madhya pradesh, Utter pradesh, Gujarat, Tamilnadu, Kerala etc. Banaskantha, Bharuch, Gandhinagar, Jamnagar, Junagadh, Kheda, Anand, Mahesana, Surat, Surendranagar, Valsad and Navasari are major area for okra.

Okra is a quite popular vegetable in India because of easy cultivation, dependable yield and adaptability to varying moisture conditions. Even within India, different names have been given in different regional languages (Chauhan, 1972).



NHB (2015)

The cytogenetic study in okra the chromosome number (2n) of *A. esculentus* L. Moench*)* have been variably reported by various scientists (Teshima; 1933, Ford; 1938, Ugale *et al*.,; 1976 and Kamalova ; 1977). The most frequently observed somatic chromosome number is 2n=130, although Dutta and Naug (1968) have suggested that the chromosome numbers 2n=72, 108, 120, 132 and 144 are in regular series of polyploids with n=12 for okra.

Okra is mainly multiplied by seeds and has duration of 90-100 days. It is generally an annual plant. Its stem is robust, erect, and variable in branching and varying from 0.5 to 4.0 meters in height. Leaves are alternate and usually palatably five lobed, whereas the flower is auxiliary and solitary. The fruit is a capsule and grows quickly after flowering. The okra pods are harvested when immature and high in mucilage and generally there are ten picking in okra crop. Okra plants continue to flower and to fruit for an indefinite time, depending upon the variety, season, soil moisture and fertility. Okra requires a long, warm and humid growing period. It can be successfully grown in hot

humid areas. For normal growth and development, a temperature between 24°C and 28°C is preferred. It is grown on sandy to clay soils but due to its well-developed tap root system, relatively light, well-drained, rich soils are ideal.

The seeds are dicotyledonous and they vary in shape; roundness, kidney or spherical with epigeal germination (Hamon *et al.,* 1991; Ariyo, 1993). Camciuc and his co-workers (1998) have suggested that the okra seed are most often used due to its superior nutritional quality. Rubatzky and Yamaguchi (1997) reported that the seed is a rich source of protein and oil. They have also reported that okra seed oil contains cyclopropenoid fatty acids which cause some toxicity concerns and is used as a substitute for coffee in some countries. Karakottsides and Constantinides (1975) found that the protein efficiency ratio of okra seed flour heated at 130ºC for 3hr was not different from the non heated flour, indicating the absence of anti-nutritional factors. They have also suggested that the amino acid composition of gumbo seed protein is similar to that of soybean and the protein efficiency ratio is higher than that of soybean. Okra (*A. esculentus*) seed oil has a similar composition to that of cotton seed oil and it may be used as a substitute for cotton seed oil in pharmaceutical formulations (Sengupta *et al*., 1974).The high percentage of linoleic acid (42%) makes okra seed oil desirable and the amino acid pattern of the protein renders it an adequate supplement to legume or cereal based diets (Savello *et al*., 1982). Mature okra seed has a harsh flavour, it can be improved by processing.

Okra provides an important source of nutraceutical such as vitamins, calcium, potassium and other mineral matters, which are often lacking in the diet of developing countries (IBPGR, 1990).The composition of 100g edible portion of okra seeds contain moisture 89.6 g, protein 1.9 g, carbohydrates 6.4 g, fat 0.2 g, calcium 66 mg, fibre 1.2 g, iron 0.35 mg, calories 35, potassium 103 mg, phosphorus 56 mg, thiamine 0.07 mg, sodium 6.9 mg, nicotinic acid 0.6 mg, sulphur 30 mg, vitamin C 13 mg, riboflavin 0.1 mg, magnesium 53 mg, oxalic acid 8 mg and copper 0.19 mg (Gopalan *et al.,* 2007).

The seed oil was found to contain high levels of unsaturated fatty acids, especially oleic (up to 24.89%) and linoleic (up to 42.78%). The okra seed oil can be classified in the oleic-linoleic acid group. The dominant saturated acid as palmitic (up to 25.79%). The okra seeds were also founded to contain high levels of crude protein (24.85%). The content of in saponifiables is 1.53% (Ndangui *et.al*, 2010).Okra cheese prepared from mature okra seed and bakery products in which wheat flour was substituted with okra seed meal at replacement levels of 25 – 100% had acceptable sensory properties (Martin and Ruberte, 1979).

As the demand, for vegetables is rapidly increasing due to the growing human population, okra, which is currently grown mainly as a vegetable crop, has potential for cultivation as nutritional crop due to its mature pods contain high quantity of seeds containing considerable amount of various nutrients which could be characterized and utilized for commercial purposes. Generally, fruits and vegetables have shown the basic useful properties

especially in providing an excellent health and nutritional qualities in the area of prevention and delay in the onset of chronic diseases and the provision of vitamins and enzymes necessary for proper body function.

In crop improvement program, availability of sufficient genetic variability is of immense importance. In a crop like okra, the data generated through molecular tools such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), and biochemical techniques like isozymes and protein profiling through Polyacrylamide Gel Electrophoresis will be useful for characterization and identification of various okra genotypes. In particular these technologies have important applications in plant biotechnology, plant breeding, seed production, and seed testing program.

It is possible to characterize / identify a variety more accurately than taxonomically by analysis of seed and seedling protein, isozymes, and DNA based techniques like, Randomly Amplified polymorphic DNA (RAPD) and Simple Sequence Repeats (SSR). Because of high mucilage content of okra it is very difficult to extract protein and nucleic acid. Keeping in view the above facts the present investigation **"Characterization of Okra (*Abelmoschus esculentus* L. Moench) Genotypes through Biochemical and Molecular markers"** is planned to study the genetic diversity among genotypes of okra with following objectives.

1. To examine okra seed for variation in moisture, total lipid, free fatty acids, true protein and crude protein.

2. To examine okra seed for variation in protein profiling through polyacrylamide gel electrophoresis (PAGE).
3. To study isoenzymatic pattern of various okra seedlings using polyacrylamide gel electrophoresis (PAGE).
4. To assess molecular characterization of different okra genotypes using various molecular marker techniques *viz.,* RAPD and SSR.

CHAPTER II

REVIEW OF LITERATURE

The current literature represents summary of research work related to morphological, biochemical and molecular markers among okra cultivars. To fill up the gap, wherever it was necessary, the recent reviews on related same species or other vegetable crop have been included and have been reviewed and highlighted under the following sub-headings.

2.1 GEOGRAPHICAL ORIGIN AND DISTRIBUTION:

Okra (*Abelmoschus esculentus* L.) is found all around the world from Mediterranean to equatorial areas as seen from the geographical distribution of cultivated and wild species shown in Fig 2.1.

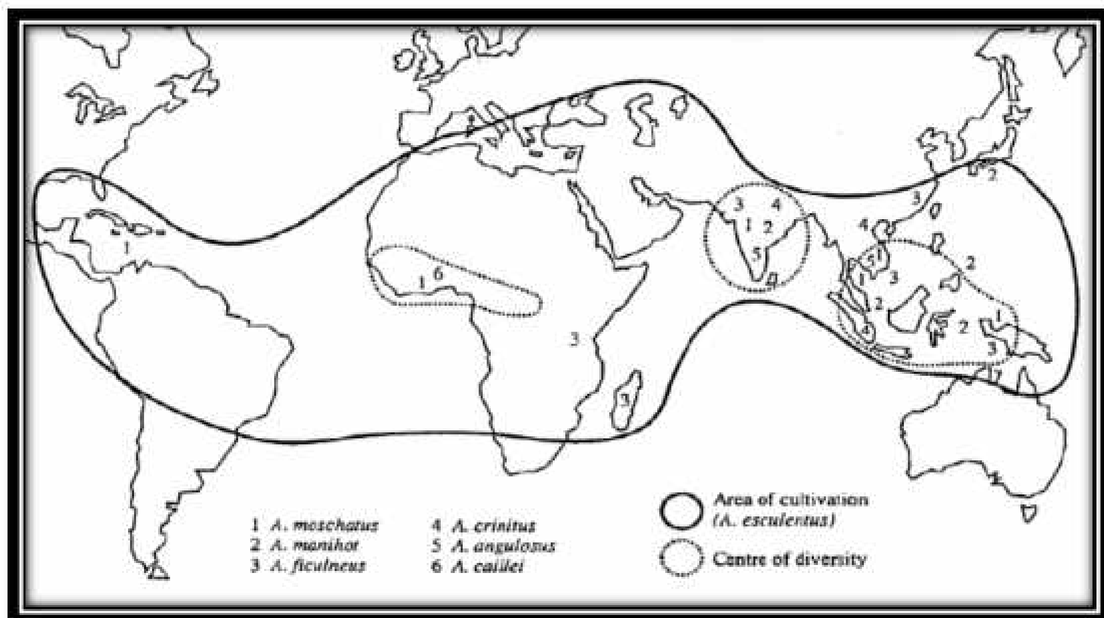


Fig 2.1 Geographical distribution of cultivated and wild species.

Cultivated and wild species clearly showed overlapping in Southeast Asia, which is considered as the centre of diversity.

The spread of the other species is the result of their introduction to America and Africa. There are two hypotheses concerning the geographical origin of *A. esculentus*. Some authors argue that one putative ancestor (*A. tuberculatus*) is native to Uttar Pradesh in northern India, suggesting that the species originated from this geographic area. Others, on the basis of ancient cultivation in East Africa and the presence of the other putative ancestor (*A. ficulneus*), suggest that the area of domestication is north Egypt or Ethiopia, but no definitive proof is available till today. *A. caillei*, only found in West Africa, it is difficult to suggest an origin outside. Its origin through hybridization with *A. manihot* is difficult to accept even if its presence, mentioned in the Flora of West Africa (Hutchinson and Dalzid, 1958).

Eight *Abelmoschus* species occur in India. Out of these, *A. esculentus* is the only known cultivated species. *Abelmoschus moschatus* occurs as wild species and is also cultivated for its aromatic seeds, while the rest six are truly wild types. The wild species occupy diverse habitats. The species *A. ficulneus* and *A. tuberculatus* are spread over the semi-arid areas in north and northwestern India; *A. crinitus* and *A. manihot* (*tetraphyllus* and *pungens* types) in tarai range and lower Himalayas; *A. manihot* (*tetraphyllus* types), *A. angulosus*, and *A. moschatus* in western and eastern ghats; and *A. crinitus* and *A. manihot* (mostly *pungens* types) in the northeastern region depicts their broad range of distribution in different phyto-geographical regions of the country. Intra as well as interspecific variations do exist in different phyto-geographic areas. Existence of different *Abelmoschus* species in different areas

of India observed in a recent survey (Bisht and Bhat, 2006) is presented in the (Table 2.1).

Table 2.1: Different species of okra

Sr no.	Species	Distribution
1	*A. angulosus*	Tamil Nadu, Kerala
2	*A. cancellatus*	Uttaranchal, Himachal Pradesh, Uttar Pradesh, Orissa
3	*A. crinitur*s	Uttaranchal, Madhya Pradesh, Orissa
4	*A. ficulneus*	Jammu & Kashmir, Rajasthan, Madhya Pradesh, Chhattisgarh, Maharashtra, Tamil Nadu, Andhra Pradesh, Uttar Pradesh
5	*A. manihot*s sp. tetraphyllus var. *tetraphyllus*	Uttar Pradesh, Rajasthan, Madhya Pradesh, Maharashtra, Orissa, Chhattisgarh
6	*A. manihots sp. Tetraphyllus* var. *Pungens*	Uttaranchal, Himachal Pradesh, Jammu & Kashmir, Assam, Andaman & Nicrobar Islands
7	*A. moschatus* ssp. Moschatus	Uttaranchal, Orissa, Kerala, Karnataka, Andaman & Nicobar Islands
8	*A. moschatus* ssp. *Tuberosus*	Kerala and parts of Western Ghats in Tamil Nadu
9	*A. tuberculatus*	Uttar Pradesh, Rajasthan, Madhya Pradesh, Maharashtra

(Bisht and Bhat, 2006)

2.2 MORPHOLOGICAL ATTRIBUTES:

2.2.1 Plant height

The performance of different okra (*Abelmoschus esculentus* L.) cultivars under the agro-climatic condition of Dera Ismail Khan was studied by Rahman *et al.* (2012) and they recorded the plant height was ranged between 64.17 cm (Sharmeeli) to 96.83 cm (Puja).

The plant height of okra ranged between 38 -147.65 cm in 163 okra genotypes during the study on the genetic variability, nature of association among different yield attributes and their direct and indirect contribution towards yield. (Salimath *et al.* 2011).

Rahman and Akter (2012) applied five different doses of NPK fertilizers on okra plant and the highest plant height reported in T_1 treatment (N_1P_1K, 2 kg cow dung, 65 gm urea, 150 gm TSP and 105 gm MP) 63.11 cm in Arka Anamika cultivar.

Talib *et al.*, (2012) studied role of different physico-chemical characters of nine cultivars of okra plant among this the highest plant height was observed for Diksha (132.7cm), while the lowest plant height was with Super star (101 cm).

2.2.2 Fruit length (cm)

Kasrawi *et al.*, (2007) observed the fruit length of okra was ranged between 9 to 23 cm.

Weerasinghe *et al.,* (2010) studied okra fruits and reported 15-20 cm fruit length. The data revealed that mean fruit length did not show any

significant difference in relation to the different fertilizer treatments throughout the growing period.

Kabir and Pillu (2011) observed that the okra fruit length was maximum 11.83 cm in Shagun followed by 11.75 cm in Arka Anamika and 11.16 cm in Sinnova.

Salimath *et al.,* (2011) observed that the fruit length of okra ranged between 9.1 – 17.35 cm in 163 okra genotypes during the study on the genetic variability, nature of association among different yield attributes and their direct and indirect contribution towards fruit yield.

Talib *et al*., (2012) have studied role of different physico-chemical characters of nine cultivars of okra plant. They have recorded that the maximum fruit length and minimum fruit length observed in Diksha (12.70 cm) and Pusasawani (8.90 cm), respectively.

2.2.3 Fruit girth (cm)

Tapankumar *et al*., (2009) studied performances of F_1 hybrids of okra under reduced level of chemical fertilizers (50% of recommended dose of fertilizers) with two organic manures (cow dung and neem cake) in which they found that higher fruit girth was 1.69 cm in Makhmali, while lower fruit girth was 1.51 cm in INDO-321.

Basar *et al.,* (2011) recorded the maximum fruit girth (2.13 cm) in S_6F_2 (S_6 spacing 30cm; F_2 NPK 60, 40, 40) treatment during the study on effect of fertilizer on growth parameters of okra plant.

Mishra *et al.*, (2013) have recorded the maximum fruit girth in T_6 (FYM@10 t/ha-1 100% RDF + vermicompost @5 t/ha + biofertilizer) 1.62 cm and the minimum fruit girth in T_1 (Control + FYM@10 t/ha-1) 1.39 cm.

Unah *et al.* (2014) observed the maximum fruit girth in PM_3 treatment (15 t/ha poultry manure) 2.09 cm, while the lowest observed in control (2.01 cm) during the study on effect of poultry manure on yield parameters of okra.

2.2.4 Fruit weight (gm)

Tapankumar *et al.* (2009) studied performance of F_1 hybrids of okra under reduced level of chemical fertilizers (50% of recommended dose of fertilizers) with two organic manures (cow dung and neem cake) in which they found that the highest fruit weight was found in Mahico (18 gm), while the lowest fruit weight was found in INDO-7275 (12.75 gm).

Basar *et al.,*(2011) recorded the highest fruit weight in S_4F_2 treatment (S_4 spacing 20 cm ; F_2 NPK 60, 40, 40) 21.39 gm, while the minimum fruit weight found in S_1F_1treatment (S_1 spacing 5 cm ; F_1 NPK 30, 20, 20)13.99 gm during the study on effect of fertilizer on growth parameters of okra plant.

Salimath *et al.* (2011) reported that the fruit weight of okra was ranged between 10.50 - 25.51 gm during their study on genetic variability, nature of association among different yield attributes and their direct and indirect contribution towards yield in 161 okra genotypes.

Rahman and his cowerks (2012) studied the performance of different okra (*Abelmoschus esculentus* L.) cultivars under the agro-climatic condition. They have recorded the fruit weight was ranged between 8.23 gm to 10.33 gm.

Mishra *et al.*,(2013) observed the maximum 18.70 gm and the minimum 11.62 gm weight of single fruit of okra with treatment T_6 (FYM@10 t/ha-1 100% RDF + vermicompost @5 t/ha + biofertilizer) and T_1 (Control + FYM@10 t/ha-1), respectively.

2.2.5 Fruit Volume (cm^3)

Ikrang (2014) observed that average value of volumes of the three fruits of okra was 13.28 cm^3 during the study of physical properties of some tropical fruit. The volume of a fruit was determined by liquid displacement method.

Ashish japda (2014) studies the morphological characters of okra in ten cultivars from that he reported that fruit volume is significantly higher in GAO-5 with 9.28 cm^3.

2.2.6 Fruit density (g/cm^3)

Chaudhary *et al.* (2007) recorded the highest density in T_3 (15 April) 2.05 g/cm^3, while the lowest was observed in T_1 (15 February) 1.68 g/cm^3 in the okra cultivar Bari Dherosh-1 during the effect of sowing time in okra.

Warade *et al.*, (2007) studied an effect of liquid seaweed fertilizer on yield and quality of okra, they have maximum recorded the highest fruit density in T_2 (LSF @ conc. 5%) 1.36 g/cm^3.

Ikrang (2014) reported an average value of fruit density of three fruits okra was 1.04 g/cm^3 during the study of physical properties of some tropical fruit.

El – waraky (2014) studied an effect of sowing date (15 March, 15 April, 15 May) on fruit characteristic, and noticed the higher density of okra pods in 15 April (2.2 g/cm^3) sowing date.

2.3 PHYTOCHEMICAL ATTRIBUTES:

The study of physiochemical, phytochemical and toxicity studies on gum and mucilage from plant *Abelomoschus esculentus* have been evaluated by Shankul Kumar, (2011) they have revealed from their study that the presence of carbohydrates, gums, and mucilges, proteins, phytosterols, flavanoids, tannins and phenolic compounds.

The *Abutilon* leaves were also subjected for phytochemical screening. Methanolic, petroleum ether and chloroform extracts were selected for the present study as this plant possesses several secondary metabolites like alkaloids, glycosides, phenols, steroids, terpenoids, saponins, flavanoids and proteins. The petroleum ether extract confirmed the presence of sterols, terpenoids and alkaloids. The chloroform extract showed the presence of alkaloids, sterols, flavonoids and glycosides while the methanol extract confirmed the presence of carbohydrates, flavonoids, tannins, phenolic compounds, proteins and amino acids.

Ibrahim Sani (2014) worked on phytochemical screening dried Abelmoschus *esculentus* L. fruits. The result of the qualitative phytochemical screening revealed that the fruits contain saponins, flavonoids, tannins, steroids and terpenoids, while, anthraquinone; alkaloids, phenols, glycosides and phlobatannins were not detected.

2.3 BIOCHEMICAL ATTRIBUTES:

2.3.1 Moisture

AL- Watt and Merril (1963) found the moisture content of okra seeds was 7.92 %. Wandawi (1983) reported that the moisture content of okra seeds were 6.96 % for Emerald variety and 17.66 % for Ibtaira variety.

Kushwaha *et al*., (2005) reported that the increase in moisture level 12.3, 15.8, and 18.8% with decrease in germination percentage 94.7, 93.1 and 92.9 % respectively.

El Balla *et al.,* (2011) studied the effect of moisture content on hard seedness and germination in okra. Seed moisture content within each cultivar significantly decreased with the increase in seed maturity. They have noticed higairat had the highest moisture content percentage (53.6%).On the other hand, Afreeta had the lowest moisture content (29.88%).

Mohammadi *et al*., (2012) reported the germination percentage reduced with increase in moisture content and vise-versa, moisture content also the factor that proposed has effect on seed hardness, as the seed matured and by decreasing it's moisture content percentage.

Dhruve *et al.,* (2015) analyzed the seed moisture content in ten okra cultivars, among them significantly lower moisture content was recorded with GAO-5, while higher moisture content was recorded with AOL 13-88 (6.40%) which was at par with AOL 13-90 (6.26%).

2.3.2 True protein

Arlai *et al.,* (2009) observed that the conventional okra pod contained 5.2 % protein while the organic okra contained 11.5 % protein.

Azam khan *et al.,* (2013) reported the higher protein content in genotype IN-1048 (2.57%), while 1936 (2.00%) had lower protein content in okra, the study was carried out genetic variability under nutrient uptake in okra under irrigated and wastewater ecosystem.

Dhruve *et al.,*(2015) found that true protein content was significantly the highest and the lowest protein content were recorded in GAO-5 (18.96%) and in AOL-13-88(14.05%),respectively from okra seeds.

2.3.3 Crude protein

Akenovat *et al.,* (1982) studied changes in the crude protein constituents of okra (*Abelmoschus esculentus* L.) with age, and they reported the range as 19.9 to 24.7 %, and it was decreased with age.

Gemede *et al.,* (2015) reported that Okra pod of "OPA#6"(26.16 %) accession contained significantly higher amounts of crude protein among the eight genotypes of okra.

Hardeep kaur *et al.,* (2015) studied that the effect of different seed priming treatments and priming duration on biochemical parameters of okra (*Abelmoschus esculentus* L.) as a priming treatment taken three combinations T_1,T_2 and T_3 with soaking duration 24, 30, 36, 42 and 48 at 6h interval. This investigation resulted significantly increased crude protein (16.02 %) in T_1 treatment for 24 h soaking solution.

Ogungbenle *et al*., (2015) reported the proximate and functional properties of nigerian dried seeds were evaluated in okra (*Abelmoschus esculentus* L.), the results shows crude protein content is 26.4% in dry okra seeds.

2.3.4 Total lipids and free fatty acids

Anwar and his coworkers, (2011) have stated that the seeds from two varieties namely Sabz Pari and Punjab-8 of Okra (*Hibiscus esculentus*), grown under similar environment, exhibited oil content 11.72 and 13.42%, respectively.

Manal (2015) investigate the nutritional analysis of three Egyptian cultivars of okra (*Abelmoschus esculentus* L.) seeds namely: Barady Assiut (BA) , Balady Qena (BQ), Pusa Sawani (PS) it resulted that singnificantly ($p < 0.05$) higher oil content was recorded in (23.99%) while lower seed oil content (16.06 %) was recorded in Barady Assiut (BA) .

Ogungbenle and Omosola, (2015) revealed that the most prominent of fatty acid in okra seed oil were linoleic acid (CI8:2) was the most concentrated fatty acid with the value of 30.94% followed by oleic acid (29.13%).

2.5 BIOCHEMICAL MARKERS:

Okra (*Abelmoschus esculentus* L.) is an important vegetable grown extensively in India. The mature seeds of this plant have not been exploited for human nutrition. Recently, okra seeds were suggested as a new protein source since amino acids composition is similar to that of soybeans (Karakoltsidis and Constantinides, 1975). Seed development has been studied

(Chandra and Bhatnagar, 1975, 1976) but very little information is available regarding its molecular aspects.

The ability and potential of gel electrophoresis of protein and isozymes for distinguishing crop varieties have been demonstrated by many researchers (Iwasaki *et al.,* 1982; Smith and Wych, 1986 and Halim and Saxena, 1992). Biochemical markers like seed proteins and isozymes have been used in seed purity discrimination of various crops (Agrawal, 1988; Kumar *et al*., 2007).

Isoenzymes are functionally similar forms of enzymes (Murphy *et al*., 1990). Allozymes are different forms of the same enzyme resulting from allelic variation (Crozier, 1993), which display differential mobility with electrophoretic techniques and can be detected by staining for enzyme activity (Conkle*et al*., 1982). The net charge of the protein influences its movement in an electrical field (Hartl, 1988) and other important factors influencing protein migration are its size and shape (Murphy *et al*., 1990). Biochemical studies met with considerably more success than previous studies using morphological markers. However, the numbers of genetic markers provided by isoenzyme assays are insufficient in many plant breeding applications (Tanksley, 1993).

2.5.1 Isozymes:

Isozymes analysis has been used for over 60 years for various research purposes in biology *viz.,* to delineate phylogenetic relationships, to estimate genetic variability and taxonomy, to study population genetics and

developmental biology, characterization in plant genetic resources management and plant breeding (Bretting and Widrlechner, 1995; Staub and Serquen, 1996). Isozymes have been widely used as protein markers (Bournival *et al*., 1989; Powell, 1992; Hongrun & Yun-Tzu, 1993; Isshiki *et al*., 1994; Karihaloo & Gottlieb, 1995; Lebot *et al*., 1998; Lioi *et al.,* 1998). Isozymes exhibit codominance at a locus making it possible to differentiate between heterozygotes.

Isozymes are idefined as structurally different molecular forms of an enzyme with qualitatively the same catalytic function. Isozymes originate through amino acid alterations, which cause changes in net charge, or the spatial conformation of the enzyme molecules and also, therefore, their electrophoretic mobility. After specific staining the isozyme profile of individual sample can be observed (Hadacova and Ondrej, 1972, Vallejos, 1983 and Soltis, 1989).

Patel *et al.,* (2011) analyzed peroxidase isozymes from okra cultivar and reported that the soluble protein banding pattern was more effective for cultivar identification.

2.5.2 Protein profiling:

Torkpo *et al.,* (2006) analyzed 20 okra (*Abelmoschus esculentus* L.) accessions for diversity through total and seed storage proteins. A total of 34 reproducible and easily scorable bands were observed with the number of bands per accession ranging from 1 to 21. Banding patterns of storage

proteins were the most diverse and could be used for the discrimination of okra genotypes.

Soluble protein was extracted from seeds of okra with 3% NaCl (Patel *et al.,* 2001). Zymogram of protein for each genotype showed that the banding pattern was more effective for cultivar identification of okra (*Abelmoschus esculentus* L.).

Azeez and Morakinyo (2004) studied the electrophoretic characterization of crude proteins in *Lycopersicon* and *Trichosanthes* genotypes with young leaves at 50% flowering on gel electrophoresis. The results showed that inter cultivar qualitative as well as quantitative protein bands depicted some degree of relationship among the genotypes. The degree of variation in protein bands was used as a measure of genetic divergence between *L. esculentum* genotypes and *T. cucumerina*.

Zubaida *et al.,* (2006) analyzed seed protein profiles of 54 accessions belonging to 11 species of 2 different genera (*Solanum* and *Capsicum*) of the family *Solanaceae* by SDS-PAGE. Intra and inter specific relationship was estimated using Jaccard's similarity index. A dendrogram based on UPGMA revealed the generic status of *Solanum* and *Capsicum. S. surattense* based on morphological and on protein profiles.

Dadlaniand *el al.,* (1994) reported superiority of SDS-PAGE for soluble seed protein as a reliable and very fast process to identify the variety.

2.6 MOLECULAR MARKERS

The assessment of genetic diversity within and between populations is routinely performed at the molecular level using various laboratory-based techniques such as allozyme or DNA analysis, which measure levels of variation directly. Genetic diversity may be also gauged using morphological, and biochemical characterization and evaluation:

(i) Morphological characterization does not require expensive technology but large tracts of land are often required for these experiments, making it possibly more expensive than molecular assessment. These traits are often susceptible to phenotypic plasticity; conversely, this allows assessment of diversity in the presence of environmental variation.

(ii) Biochemical analysis is based on the separation of proteins into specific banding patterns. It is a fast method which requires only small amounts of biological material. However, only a limited number of enzymes are available and thus, the resolution of diversity is limited.

(iii) Molecular analyses comprise of large variety of DNA molecular markers, which can be employed for analysis of variation. Different markers have different genetic qualities i.e. they can be dominant or co-dominant, can amplify anonymous or characterized loci, can contain expressed or non-expressed sequences, etc.

The different methods of molecular assessment differ from each other with respect to important features such as genomic abundance, level of polymorphism detected, locus specificity, reproducibility, technical

requirements and cost. Depending on the need, modifications in the techniques have been made, leading to advanced molecular markers.

Genetic polymorphism is defined as the simultaneous occurrence of a trait in the same population of two discontinuous variants or genotypes. DNA markers seem to be the best candidates for efficient evaluation and selection of plant material. Unlike protein markers, DNA markers segregate as single genes and they are not affected by the environment. DNA is easily extracted from plant materials and its analysis can be cost and labour effective. Genetic variability within a population can be accessed through:

1. The number (and percentage) of polymorphic genes in the population.
2. The number of alleles for each polymorphic gene.
3. The proportion of heterozygous loci per individual.

The term microsatellites was coined by Litt and Lutty (1989), and it also known as Simple Sequence Repeats, are sections of DNA, consisting of tandemly repeating mono-, di-, tri, tetra- or penta-nucleotide units that are arranged throughout the genomes of most eukaryotic species (Powell *et al*., 1996).

Microsatellite markers, developed from genomic libraries, can belong to either the transcribed region or the non transcribed region of the genome, and rarely is there information available regarding their functions. Microsatellite sequences are especially suited to distinguish closely related genotypes; because of their high degree of variability and hence they are

favoured in population studies (Smith and Devey, 1994) and for the identification of closely related cultivars (Vosman *et al*., 1992).

Simple sequence repeats consist of tandem repeated DNA sequences, like (AT)n surrounded with specific sequences and are mainly located in the non coding part of the genome. Polymorphisms are due to variation in the number of detected repeats. SSRs are amplified by PCR, using flanking primers. They are highly polymorphic and provide co-dominant genetic markers following Mendelian inheritance (Tautz, 1989; Russell *et al*., 1997).

Simple Sequence repeats are considered as the marker of choice for self-pollinated crops with little intra specific polymorphism (Roder *et al*., 1998). They are reproducible and it can be efficiently used by different laboratories to produce consensus data, which makes them useful for genome mapping projects and results in their successful isolation and application within many plant species (Schmidt and Heslop, 1998).

The high incidence of detectable polymorphism through changes in repeat numbers is caused by an intra molecular mutation mechanism called DNA slippage (Gupta *et al.,* 1996). The regions flanking the microsatellites are generally conserved and PCR primers relative to the flanking regions are used to amplify SSR- containing DNA fragments. The length of the amplified fragment varied according to the number of repeated residues (Ellegien, 1993).

Sawadogo *et al.,* (2009) analyzed the sixteen pairs of primers designed to amplify SSR regions of *Medicago truncatula,* and were used to amplify

genomic DNA samples of 20 different okra (*Abelmoschus esculentus*) accessions collected from different regions of Burkina Faso. Two okra accessions were different from other 18 based on molecular markers as well as morphological features of their fruits. One of the primers, MT-27 amplified unique 440 bp PCR products in these two okra accessions. This PCR product was sequenced and based on the sequence information, sequence specific primers were designed to PCR amplify the genomic DNA of all the okra accessions. These pair of primers amplified PCR product only in two okra accessions, amplification of PCR product was seen with MT -27 primer. The data indicated that cross species SSR primers developed for *Medicago truncatula* can also be used to analyzed genomic diversity in unrelated species like okra.

The cultivated diploid, *Gossypium arboretum* L., (A genome) is an invaluable genetic resource for improving modern tetraploid cotton (*G. hirsutum* L. and *G. barbadense* L.) cultivars. About 1,500 previously developed simple sequence repeat (SSR) markers, 115 genomic (BNL) and EST-derived (MUCS and MUSS) markers were used to evaluate the allelic diversity of a core panel of *G. arboretum* accessions. Outcomes of this research was useful in decreasing redundancy of effort and in constructing a core collection of *G. arboreum*, important for efficient use of this genetic resource in cotton breeding (Kantartzi *et al., 2009).*

Parentage of F_1 hybrids of cotton was verified using random amplified polymorphic DNA (RAPD) and microsatellite (SSR) assays. Out of 500 primers surveyed, 3 random and 3 EST based SSR primers were found

polymorphic between two cotton parents. Based on their results they confirmed the parentage of their true F_1 hybrids and revealed that the RAPD and SSR procedures were excellent genomic tools for parentage confirmation and hybrid determination (Asif *et al*., 2009).

Pritesh *et al*., (2010) studied phenotypic and genetic diversity of twenty five determinate and indeterminate cultivars of tomato from different geographical locations of India. Total 23 SSR primers were used in order to determine genetic identities, genetic diversity and genetic relationships among these cultivars. On an average, 40 alleles were amplified fragment sizes ranging from approximately 150 to 1000 bp.

CHAPTER III
MATERIALS AND METHODS

The present investigation is intended to analyze the morphological, biochemical and molecular characterization of okra (*Abelmoschus Esculentus* L.). The study was carried out at Main Vegetable Research Station, Department of Biochemistry and Department of Agril. Biotechnology, Anand Agricultural University, Anand during kharif 2015. The materials used and methodology adopted for the studies are described under following sub headings.

3.1 GENERAL

3.1.1 Location

Anand Agricultural University, Anand, where the present investigations were under taken, is situated on 22º- 35′ north latitude and 72 º- 55′ east longitudes and has an elevation of 45 meters above the mean sea level.

3.1.2 Climate and weather conditions

The climate of Anand in middle Gujarat region is semi-arid and tropical. Winter is mild, cool and dry, while summer is quite hot and dry. The hot temperature commences by about mid-February and ends by about middle of June. An average annual temperature for this region ranges from 34.4ºC to 40.9ºC and hottest month observed is May. Winter sets in the middle of October and continues till the middle of February. Monsoon is warm and moderately humid. It commences by the middle of June and ends by the middle of September. An average rainfall of the tract is about 864 to 870 mm.

Monsoon in this area is often erratic and uncertain, both in respect of total rainfall and its distribution.

3.1.3 Soil

The soil of the experimental site is classified as typical sandy loam locally known as "Goradu". It is alluvial in origin, deep, well drained and has fairly good moisture holding capacity. It is poor in organic matter content.

3.2 EXPERIMENTAL DETAILS

The details of the experiment are given in the following sub-heading.

3.2.1 Site

The present experiment was conducted at Main Vegetable Research Station, Department of Biochemistry, B. A. College of Agriculture and Department of Agril. Biotechnology. Anand Agricultural University, Anand.

3.2.2 Experimental Material

The experimental material consist of 30 genotypes of okra

Table 3.1: List of thirty okra genotypes:

Sr. No.	Genotypes	Sr. No.	Genotypes
1	AOL 12-52	16	GP-OK-213
2	AOL 14-32	17	GP-OK-292
3	AOL 13-94	18	GAO-5
4	AOL 09-02	19	Red okra
5	AOL 14-29	20	Pusa sawani
6	JOL 11-12	21	Parbhani kranti
7	AOL 13-141	22	AOL 13-73
8	JDNO 11-11	23	AOL 12-55
9	Kashi kranti	24	AOL 13-88
10	JDNO 11-12	25	AOL 13-112
11	AOL 13-133	26	AOL 14-08
12	JOL 09-05	27	AOL 13-90
13	AOL 14-11	28	AOL 12-59
14	JOL 13-05	29	JOL 13-07
15	GP-OK-296	30	AOL 13-144

3.2.3 Layout details

The randomized complete block design was used for present experiment. Main plot was divided in 3 blocks, each block was a replication. The block was further divided in 10 sub plots to which each genotype was allotted by complete random process.

Table 3.2: Field experiment

1	Crop	Okra
2	Treatment	30 genotypes
3	Year and season	*Kharif*, 2015
4	Design	RCBD (Randomize complete Block Design)
5	Replications	3 (Three)
6	Plot size	1.8 X 2.4 m
7	Total number of plots	90
8	Spacing	60 X 30 cm^2
9	Manures and fertilizers	N: P: K @ 100: 50: 50 kg/ha
10	Date of sowing	4th June 2015

3.2.4 Chemicals and solvents

All the chemicals, analytical and molecular grades used for biochemical and molecular work in the experiments were obtained from standard manufacturers through local dealer *viz*., Himedia, Bio-rad, Takara etc.

3.2.5 Glasswares and polywares

All glasswares and polywares were obtained from Borosil and J-Sil. All the glasswares were cleaned by using lab wash and washed with tap water

and finally rinsed with distilled water. All the plastic wares like micro tips and 2 ml eppendorf tubes were autoclaved before use.

3.3 Observations

All the observations with respect to morphological and biochemical attributes for fruits and seeds were recorded, respectively. Five plants were selected randomly, for the purpose of recording various observations are under and further used for statistical analysis.

3.4 Morphological attributes

3.4.1 Plant height at harvest (cm)

Plant height of 5 randomly selected plants was measured from the base of the stem to the apex of the central leaf at the time of harvest and average was worked out and expressed in centimeters.

3.4.2 Fruit length (cm)

Randomly 5 fruits were selected from each selected plant and length was measured by using vernire calipers and expressed in centimeters.

3.4.3 Fruit girth (cm)

Randomly 5 fruits were selected from each selected plant and fruit girth was measured and expressed in centimeters.

3.4.4 Fruit weight (g)

Randomly 5 fruits were selected from each selected plant and weighed on weighing balance and fruit weight was expressed in grams.

3.4.5 Fruit Volume (cm^3)

The fruit volumes of 5 randomly selected fruits were measured after the harvest. Volumes of fruits were measured by the water displacement

method. The fruit was placed in measuring glass cylinder filled with water and the replaced water was measured for each cultivar and data were recorded as volume of the fruit in cubic centimeter.

3.4.6 Fruit density (g/cm^3)

Fruit density was calculated by ratio of fruit weight to the fruit volume for all the genotypes.

$$\text{Fruit density (g/cm}^3\text{)} = \frac{\text{Fruit weight}}{\text{Fruit volume}}$$

3.5 Phytochemical Attributes

Preliminary phytochemical screening was carried out to find the presence of the active chemical constituents in distilled water extract such as tannins, alkaloids, Flavanoids, Glycosides, and Terpenoids (Salkouski test). In general, tests for the presence of phytochemical compounds involved the addition of appropriate chemical reagent(s) to the extract in test tubes. The mixture was then shaken and/or heated as the case may be (Table 3.3).

Table: 3.3: List of Phytochemicals and methods

Sr. No.	Phytochemical	Reagents	Reaction	Observations
1	Alkaloids (N Tamilselvi ,2012)	1 ml of Dragendorff's reagent	A small portion of crude extract was dissolved in 5ml of 1% hydrochloric acid, filtered and tested with Dragendorff's reagent and Mayer's reagent Separately.	Any precipitate or turbidity with the reagents suggests the presence of alkaloids.
2	Flavanoids (Roopashree, *et al.*, 2008).	sodium hydroxide	(Alkaline Reagent Test). Extracts were treated with few drops of sodium hydroxide solution.	Formation of intense yellow colour, which becomes colourless on addition of dilute acid, indicates the presence of flavonoids.
3	Glycosides (Shankul kumar ,2014)	Glacial acetic acid ferric chloride	Five ml of each extracts was treated with 2 ml of glacial acetic acid containing one drop of ferric chloride solution. This was under layer with 1 ml of concentrated sulphuric acid.	A brown ring of the interface indicates a deoxysugar characteristic of cardenolides. A violet ring may appear below the brown ring, while in the acetic acid layer, a greenish ring may form just gradually throughout thin layer.
4	Tannins (N Tamilselvi ,2012)	10% ferric chloride	2 ml of sample in test tubes + 1 ml of 10% ferric chloride solution is added, then observed	The formation of precipitates in solution and also it show the colour are change as bluish or brownish-green precipitate it indicate the presence of tannins.
5	Terpenoids (Shankul kumar ,2014)	Chloroform H_2SO_4	Five ml of each extract was mixed in 2 ml of chloroform, and concentrated H_2SO_4 (3 ml) was carefully added to form a layer.	A reddish brown colouration of the interface was formed to show the presence of terpenoids.

3.6 Biochemical Attributes

3.6.1 Moisture

Moisture content of okra seed was determined by drying the weighed sample of okra seed at 105˚ C in hot air oven for 3 hours and the loss of weight was expressed as moisture content (A.O.A.C., 2000). One gram seed sample from each variety was taken in pre-weighed petriplates and calculate the moisture by the following formula;

$$\text{Moisture } \% = \frac{(\text{Fresh weight} - \text{Dry weight})}{\text{Fresh weight}} \text{ X } 100$$

3.6.2 Crude protein

Crude protein content was determined by the micro-kjeldahl method used from A.O.A.C (1965).

The seed powder (1 gm) was weighted and transfer it into a digestion flask add 1 gm of digestion mixture (99.0 gm of K_2So_4 ,4.1 gm of HgO and 0.8 g of $CuSo_4$) and 2 ml of concentrated sulfuric acid. Digest it until the solution is colorless (approx.40 min. at 150 to 200 ˚c.) After cooling add minimum quantity of water to dissolve solids and allow to cool. Add 10 ml sodium hydroxide –sodium thiosulphate solution, on other side take 10 ml of boric acid solution into a 100 ml Erlenmeyer flask both the tube and flask put into micro-kjeldahl machine run protocol (brown to blue colour observed) after 4 min. take out from instrument. Add 1 drop of methyl indicator in flask titrate against 0.02N H_2SO_4 .The sample calculated using the following (micro kjedahl method).

$$\% N = \frac{X - \text{blank} \times 0.00014 \times \text{dilution factor} \times Y \times 100}{}$$

X: Titrate value, Y: weight of sample (g)

The crude protein content was calculated by multiplying % N with a factor 6.25.

3.6.3 True protein

Protein content was determined by the method developed by Lowry *et al.,*(1951).One gram sample was weighed and homogenized in five ml 0.1 N NaOH and filtered through whatman No.1 filter. The sample extracts (2.5 ml) was taken and made to 3.0 ml volume with distilled water. Five ml of alkaline copper reagent C was added {(A) Two percent sodium carbonate in 0.1N sodium hydroxide. (B) 0.5 % copper sulphate in 10 % sodium potassium tartrate. (C) Prepared by mixing 50 ml of reagent A with 1.0 ml reagent B. This reagent was prepared fresh at the time of use} and mixed well. The content was allowed to stand for 10 minutes at room temperature followed by addition of 0.5 ml Folin-Ciocalteau reagent (diluted with water 1:1 v/v). The content was kept for 30 minutes at room temperature and the absorbance was measured at 750 nm. The protein content was calculated using bovine serum albumin as standard range from 50 – 300 µg.

$$\text{True protein } \% = \text{Graph factor} \times \frac{\text{Sample reading}}{\text{Weight of sample}} \times \frac{\text{Total volume}}{\text{Taken volume}} \times 10^{-4}$$

3.6.4 Total lipids

Total okra seed lipid was estimated by soxhlet extraction method. 5g of okra seeds were extracted for 8 h with petroleum ether 60 – 80 ^{0}C (A.O.A.C., 1965). The solvent was distilled out and the flasks were then transferred to

oven maintained at 80 ^{0}C for 24 h. The flasks were removed from oven and kept in desiccator until it comes to room temperature. The flasks were weighed and per cent oil was calculated as,

$$\text{Oil Percentage} = \frac{\text{Weight of flask+oil}-\text{weight of flask}}{\text{Weight of Sample (g)}} \times 100$$

3.6.5 FREE FATTY ACIDS

Free fatty acids was analysed & recorded according to Cox & Pearson, 1962. Weigh 0.2 gram of oil in a 150 ml conical flask and add 2 ml of fat solvent, 150 µl 0.1 N KOH and add few drops of phenolphthalein solution. Mix thoroughly and titrate with 0.1 N KOH until faint pink colour persists. Record the observations. Calculate the acid value (AV) and free fatty acid (%FFA)

$$AV = \frac{\text{Titrate value} \times \text{ ml of KOH } \times N \times 56.1}{\text{weight of sample (gm)}}$$

$$FFA\% = AV \times 5.03$$

3.7 Biochemical Markers

3.7.1 Isozymes pattern of enzymes

A. **Polyphenol oxidase**
B. **Peroxidase**
C. **Esterase**

3.7.1.1 Extraction of Enzymes for Electrophoresis

One gram of okra seedlings was homogenized with a pre-chilled mortar and pestle under ice cold condition in 500 µl of extraction buffer, containing 0.1 M Sodium phosphate buffer (pH 7.2) with 1%

polyvinylpyrolidone (PVP). The homogenates were centrifuged at 10,000 rpm for 20 min and supernatants were used for characterization of isozymes (peroxidase and poly phenol oxidase).

Electrophoresis was carried out on vertical electrophoresis unit using 1 mm gel. Electrophoresis was performed at 30 mA for 1.5-2.0 hrs at 4 ^{0}C until tracking dye moved at bottom. Enzyme extracts (100 µg protein) were loaded for each isozyme and mixed with 2 µl tracking dye. Protein concentration was determined by estimating soluble protein content by Lowry's method.

Table 3.4: Preparation of gel for isozyme

Reagents	10% Running gel (40 ml)	5% Stacking gel (10 ml)
Double Distilled Water	15.9 ml	6.84 ml
30% Acrylamide	13.3 ml	1.7 ml
Tris buffer pH 8.8	10 ml	1.25 ml
10% APS	400 µl	100 µl
TEMED	16 µl	10 µl

3.7.1.2 Procedure

a) Polyphenol oxidase

Polyphenol oxidase isozymes were separated on 10 % non- denaturing polyacrylamide gels. Isoforms of PPO were visualized by incubating the gel in 0.197 g of DL- Dihydroxyphenyl alanine in dark at 30^0 C for one hr. (Smith ,1970).

b) Peroxidase

Peroxidase isozymes were separated on 10% non- denaturing polyacrylamide gels. Isoforms of POX were visualized by incubating the gel in 100 ml Sodium phosphate buffer (0.025 M, pH 6.0) in dark for 10 minutes. After that the gels were transferred into 100 ml of 0.025 M Sodium phosphate buffer (pH 6.0) containing 100 µl of 30 % H_2O_2 and kept for 5-7 min in dark with gentle shaking. After this, o-dianisidine (50 mg dissolved in 1 ml of methanol) was added and kept in dark with occasional shaking until bands appeared (Jayraman,1987).

c) Esterase

Esterase enzymes were separated on 10% non-denaturing polyacylamide gels. Isoforms of esterase were visualized by incubating the gel in solution (Na_2HPO_4 and NaH_2PO_4 with adding fast blue RR dye (0.1 gram in 1 ml methanol) and alpha napthyl acetate all dissolve in 100 ml distilled water) for 15 minutes in dark condition after that observe the bands(Reddy ,1971).

3.7.2 SDS -PAGE analysis

Okra seed powder from all the varieties were homogenized with 1 ml of 0.2 M phosphate buffer pH 7.4 followed by 0.1 ml 10% SDS solution and 250 µl of β-Mercaptoethanol. Once extract was centrifuged at 4^0 C for 10 min at 10000 rpm, the clean supernatant was used for SDS-PAGE protein banding pattern analysis as per the method described by Laemmli (1970).

Proteins were separated on 12 % SDS polyacrylamide gel. The gel was washed to remove excess of SDS (Sodium Dodecyl Sulphate) and stained for overnight. Staining solution contained 0.1% Commassie brilliant blue R-250 in methanol: acetic acid and double distilled water with the ratio of 40:10:50. The gel was destained by using methanol: acetic acid and double distilled water without dye.

Table: - 3.5 Preparation of stock solutions for SDS-PAGE

Sr. No	Solution	Method of preparation
1	Acrylamide/ Bis-acrylamide solution (30%), 100ml	Dissolve 29.2g of Acrylamide and 0.8g of Bis-acrylamide in 50 ml deionised water. Adjust final volume to 100ml. Heat the solution to 37˚C to dissolve the chemicals. Filter the solution and store the filtered solution in amber colored bottles at room temperature.
2	Tris-HCL buffer, 1.5M (pH 8.8), 100ml	Dissolve Tris-base 18.17g in 50ml Millipore water. Adjust the pH to 8.8 with concentrated HCl. Adjust final volume to 100ml. Dispense into reagent bottle and stored at 4˚C
3	Tris-HCL buffer, 0.5M (pH 6.8), 100ml	Dissolve Tris-base 6.0579g in 50ml Millipore water. Adjust the pH to 6.8 with concentrated HCl. Adjust final volume to 100ml. Dispense into reagent bottle and stored at 4˚C
4	SDS (Sodium Dodecyl/ Lauryl sulphate) 10%, 100ml	Dissolve 10g of SDS in 50ml Millipore water. Adjust final volume to 100ml. Dispense into reagent bottle and stored at room temperature.
5	10% Ammonium per sulphate (APS)	Dissolve 100 mg APS in 1 ml of distilled water. Prepare fresh at the time of gel casting.
6	TEMED	TEMED (Electrophoresis grade) is used directly
7	Electrode buffer (pH 8.3)	Dissolve 3 g 0.025M Tris base and 14.4 g 0.192M glycine in distilled water and finally adjusted to 1000 ml. For SDS-PAGE 10 ml of 10% SDS is added and finally volume is made up to 1000 ml.
8	Gel loading dye (1X)	Tris Cl (pH 6.8) 50mM β- mercaptoethanol 100mM 2% SDS (For SDS-PAGE) 0.1% Bromophenol blue 10% Glycerol β- mercaptoethanol is added freshly
9	Commassie Brilliant Blue staining solution	0.25 % Commassie brilliant blue R-250 50 % Methanol 10 % Glacial acetic acid Methanol, acetic acid solution was prepared. Commassie brilliant blue was dissolved in it and filtered with Whatman filter No. 1
10	Destaining solution	50 % Methanol 10 % Glacial acetic acid

Table 3.6: Composition of Resolving and Stacking Gels

Resolving gel (12%)			Stacking gel (5%)		
	Components	**15 ml**		**Components**	**5ml**
1	H_2O	4.9 ml	1	H_2O	3.4 ml
2	30 % ACRYLAMIDE	6.0 ml	2	30 % ACRYLAMIDE	0.8 ml
3	1.5 M TRIS (pH 8.8)	3.8 ml	3	1 M TRIS (pH 6.8)	0.63 ml
4	10 % SDS	0.150 ml	4	10 % SDS	0.05 ml
5	10 % APS	0.150 ml	5	10 % APS	0.05 ml
6	TEMED	0.01 ml	6	TEMED	0.01 ml

3.8 Isolation and Purification of Genomic DNA:

Table 3.7: Preparation of stock solutions for DNA extraction

Sr. No	Solution	Method of preparation
1	1M Tris HCl (pH 8.0)	Dissolve 12.11g Tris base (Merck) in 80 ml distilled water. Adjust pH to 8.0 by adding concentrated HCl. Adjust volume to 100 ml. Dispense to reagent bottle and sterilize by autoclaving.
2	0.5M EDTA (pH 8.0)	Dissolve 7.306 g EDTA di Sodium salt (SRL) 80 ml distilleed water. Adjust pH to 8.0 by adding NaOH pellets. Adjust volume to 100 ml. Dispense to reagent bottle and sterilize by autoclaving.
3	5M NaCl	Weigh 29.92 g NaCl (Loba chem) add 50 ml of distilled water and mix well. When the salts get completely dissolved, adjust the final volume to 100 ml. Dispense in to reagent bottle and autoclave.
4	70% Ethanol, 500 ml	Take 360 ml of ethanol; mix with 140 ml of distilled water. Dispense to reagent bottle and store at 4^0C.
5	Chloroform: Isoamyl alcohol (24:1), 500 ml	Measure 480 ml of chloroform and 20 ml of isoamyl alcohol. Mix well and store in reagent bottle in room temperature.
6	Ethidium Bromide (10 mgl^{-1}), 10 ml	Add 0.1 g Ethidium Bromide to 10 ml of distilled water. Keep on magnetic stirrer to ensure that the dye has dissolved completely. Dispense to amber colored reagent bottle and store at 4^0C.

Table 3.8: Preparation of buffers for DNA extraction

Sr. No	Buffer	Method of preparation
1	CTAB Extraction buffer (4%), 10 ml	Measure 1 ml of 1M Tris HCl (pH 8.0), 2.8 ml of 5M NaCl, and 1 ml of 0.5 M EDTA (pH 8.0). Mix with about 4 ml of hot distilled water, add 0.4 g (W/V) CTAB (AMRESCO) and 0.1 g (W/V) PVP (AMRESCO) to this. Dispense to reagent bottle. Just before use, add 100 µl (1%) β-mercaptoethanol.
2	TE buffer (0.1mM), 100 ml 10mM Tris HCl (pH 8.0) 0.1mM EDTA(pH 8.0)	Take 1 ml of Tris HCl (1M), 200 µl of EDTA (0.5M). Mix with 99 ml of sterile distilled water taken in a reagent bottle, mix thoroughly, autoclave and store at room temperature.
3	TBE buffer 5X (1 liter)	Weigh 54 g of Tris base, Add 27.5 g of boric acid, 20 ml of 0.5M EDTA (pH 8.0) and around 450 ml distilled water. Dissolve the salt and adjust the volume to 1 liter.

3.8.2 Protocol for Genomic DNA extraction:

- Total DNA was extracted from the leaves by Cetyltrimethyl ammonium bromide (CTAB) method (Murray and Thompson, 1980) with some modifications as follows:
- The leaves of okra genotypes were collected from seedling and utilized for DNA extraction.
- Weighing 300 mg fruit sample from each genotype was grinded in liquid nitrogen using mortar and pestle.
- Pre-warmed (65°C) CTAB buffer (1 ml) containing 1% (v/v) β-mercaptoethanol (added freshly) and was added to each micro centrifuge tube (2 ml) and vortexed to mix.

- The tube was incubated at 65°C for 1 hr with frequent swirling. An equal volume of chloroform : isoamylalcohol (24:1) (chilled) was added and centrifuged at 10,000 rpm (Eppendorph 5810 R) and 4°C for 10 min to separate the phases.
- The supernatant was carefully decanted and transferred to a new tube.
- The above steps, beginning with the addition of chloroform: isoamylalcohol (24:1) and ending with decanting of supernatant, were repeated twice.
- The supernatant was precipitated with double volume of absolute ethanol for overnight at 4ºC. The precipitated nucleic acids were collected and washed once with the 70% ethanol and once with 80% ethanol. (DNA is very vulnerable to fragmentation at this step so the tubes should not be shaken vigorously).
- The pellets were air dried and resuspended in 100 µl of TE buffer (10 mM Tris-HCl; pH 8.0, 0.1mM EDTA, pH 8.0).
- DNase free RNase A (Fermentas, USA) 2 µl was added to the dissolved DNA stock and incubated in a water bath at 37ºC for 1 hour followed by 60ºC for 10 minutes for enzyme inactivation. The samples were stored at -20ºC deep freeze for long-term usage.

3.8.3 Purity and Quantification test of DNA

Spectrophotometry was performed to determine DNA concentration by using Nanodrop N.D.1000 (Software V.3.3.0, Thermo Scientific, USA) at absorbance ratio 260/280 nm and the quality of obtained DNA was checked

on 0.8% agarose gel. Dilution of 50 ng/µl working solution was prepared from the stock solution of the isolated DNAs.

3.8.4 Procedure:

The 1.0 µl of DNA sample was loaded into the well of Nanodrop Spectrophotometer (Thermo Scientific, U.S.A.) and the concentration of DNA and absorbance at 260 nm and 280 nm were measured and the A260/A280 ratio was automatically calculated by the software.

3.9.1 Randomly Amplified Polymorphic DNA (RAPD):

3.9.1.1 PCR Components:

a) PCR buffer (10 x)

b) Primer (10 pmoles/µl)

c) dNTPs (2.5 mM)

d) Taq DNA polymerase (3U/µl),

e) Template DNA (20 ng/µl)

Table 3.9: List of RAPD primers

Sr. No.	Primer Series	Sequence	GC content (%)
1	OPC8	5′-TGGACCGGTG- 3′	60
2	OPC19	5′-GTTGCCAGCC-3′	70
3	OPX 17	5′-GACACGGACC-3′	70
4	OPY 02	5′-CATCGCCGCA-3′	70
5	UBC 509	5′-ACAGAGACTG-3′	50
6	OPY 04	5′-GGCTGCAATG-3′	60
7	OPAE 15	5′-TGCCTGGACC-3′	70
8	UBC 465	5′-GGTCAGGGCT-3′	70

3.9.4.2 Master Mix preparation

Since the pipetting of small volumes is difficult and often inaccurate, a master mix was prepared where constituents common to all the reactions are combined in one tube multiplying the volume for one reaction with total number of samples. Later, the appropriate amount of master mix was dispensed to each tube and template DNA was added separately in each tube. PCR reactions for RAPD were carried out in a reaction volume of 15µl using the method given by Singh *et al*., (2006) with some minor modifications. The genomic DNA was amplified using random primers of OPA, OPB, OPC, OPY and UBC, OPAE, OPM series.

Table 3.10: Component for PCR mixture for RAPD

Sr.No.	Reagents	Volume
1.	PCR buffer (10 x) with 15 mM MgCl2	2.5 µl
2.	Primer (10 pmoles/µl)	0.5 µl
3.	DNTPs mix (2.5 mM each)	0.5 µl
4.	Taq DNA polymerase (3U/µl)	0.5 µl
5.	Template DNA (20ng/µl)	0.5 µl
6.	Sterile distilled water	1.5 µl
7.	Nuclease free water	19.0 µl
8.	Total	25.0 µl

As per the above cocktail Millipore sterilized water was added first followed by addition of Taq Buffer A (10X Tris with 15mM MgCl2), dNTPs, Taq DNA polymerase ,primer in sequence and finally the template DNA. The reagents were mixed gently by tapping against the tube and short spinning (~14,000 rpm for 30 seconds). The tubes were then placed in the Thermal for cyclic amplification (Table-3.11).

Table 3.11: RAPD - PCR conditions (Singh *et al.*, 2006):

	Step	Temperature (ºC)	Duration
1		94	5.0 min
2	Denaturation	94	1.0 min
3	Annealing	38	1.0 min
4	Extension	72 (35 times to step 2)	1.5 min
5	Final extension	72	5.0 min
6	Hold	4	--

3.9.1.3 Electrophoresis

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

3.9.2.3.1 Chemicals used:

Agarose (Invitrogen, Brazil)

5 X Tris Borate EDTA (TBE) buffer pH 8.0

Ethidium bromide (10 mg/ml)

100 bp DNA ladder (Fermentas, USA)

Agarose gel of 1.5 % concentration was prepared in 1X TBE (1.5 g agarose in 100 ml 1X TBE and 4µl Ethidium bromide 10mg/ml stock). PCR amplified products (9 µl and 1 µl 6X loading dye) were loaded into the wells. The molecular weight marker, 100bp ladders were used for band sizing. The electrophoresis was conducted at a constant voltage of 60 V to separate the amplified bands. The separated bands were visualized under UV transilluminator (GeNie, Bangalore, India) and photographed using gel documentation system (SYNGENE, USA).

3.9.2 SIMPLE SEQUENCE REPEATS (SSR)

The genomic DNA was amplified using primers listed in Table 3.11. PCR reactions for SSR were carried out in a reaction volume of 25 μl using method given by Sawadogo *et al.,* (2009) with minor modifications.

EST-SSR markers were synthesized by using transcription data of okra against YVMV which was conducted at Anand Agricultural University in 2013. The algorithm used for perfect and imperfect microsatellite searches was written in Perl script. The script instructions perform readings on fasta files, searching all possible arrangements in each database sequence. Several instructions in the algorithm used in SSR Locator resemble those from MISA (Thiel *et al.,* 2003). The MISA (MIcroSAtellite search tool) was configured to locate a minimum of 20>bp SSRs: monomers(x10), 2-mers(x6), 3-mers(x6), 4-mers(x5), 5-mers(x5), 6-mers(x5). The sequences in fasta format were uploaded to online software BatchPrimer3 v1.0 which synthesized primers from sequences with default parameters.

3.9.2.1 Master mix preparation

All the PCR reactions were carried out in 200 μl thin walled PCR tubes. Cocktail for PCR reaction was prepared by adding 10X Standard Taq Buffer with $MgCl_2$ (BioLabs, UK) followed by forward and reverse primers, dNTPs, Taq DNA polymerase (BioLabs, UK) and template DNA. The reagents were mixed by gently tapping against the tube and brief spin (~3,000 rpm for 30 seconds). The tubes were then placed in the Thermal Cycler (Applied Biosystems Veriti, Foster City, CA, USA) for cyclic amplification.

Table 3.12: List of SSR Primers

Sr. No	Primers	F/R	Primer sequences (‘5 – 3)	Annealing temperature (˚C)
1	OK-1	F	TATGGGGAAAACTTGAATACC	51
		R	GCATACTTTCACCCTCCA	
2	OK-5	F	CTCTCCTTCACCTTCACTTTC	52
		R	GCTGTCGATGATGGGTATTT	
3	OK-3	F	CTCAACAGTGAAATGGAAAAA	52
		R	CGAAGAAGAAGAAAAGGAGAA	
4	OK-6	F	AACCCAGTTAGCATTCTCTTT	50
		R	AGGCACTCAAGTTTTCTTTTT	
5	OK-9	F	AGTGGAAAACATCATCAAGG	50
		R	AAAAGTAAGGCTGACCAAAAG	
6	OK-10	F	ACCTCCTCGTAGGGAAGAA	51
		R	CTCCAATGCTAAATCATCAAC	
7	OK-13	F	TTCGGCTTTTGTCTGATG	51
		R	TACGGGTGAAAACGAAGA	
8	OK-16	F	GGTACTTTATCGGGATGATTG	50
		R	ATTCTTTCTTGTTTTGCCTCT	
9	OK-18	F	CAGAAATCTTCATTCATCTCTTT	51
		R	ATCATCGTTCCTCATTTCC	
10	OK-21	F	CAAAGCATCGGAAACAAC	50
		R	AGACGAAGATGTAAAACGACA	
11	OK-22	F	CAAAGCATCGGAAACAAC	50
		R	AGACGAAGATGTAAAACGACA	
12	OK-23	F	GAAATTGAAACCCCAGAAA	50
		R	CTTTCTTTGGAGTAAGGAAGTG	
13	OK-24	F	ATCTGTCTGCCGTTATTCTGT	50
		R	AGCAGAGATGTATGGTGAGG	
14	OK-27	F	ACAATGATCTCGGCTTACA	51.5
		R	CCACCCACTCCTCATATTC	
15	OK-53	F	TCACCAGCATCATCATCA	52
		R	ATCACCATTTCTTGAACCAC	

Table 3.13: Component for PCR mixture for SSR

Sr. No.	Reagents	Volume
1	10X Standard Taq Buffer with $MgCl_2$ (BioLabs, UK)	2.5 µl
2	Forward Primer (10 p moles/µl)(MWG biotech, Germany)	0.5 µl
3	Reveres Primer (10 p moles/µl) (MWG biotech, Germany)	0.5 µl
4	dNTPs (2.5mM each) (BioLabs, UK)	0.5 µl
5	Taq DNA Polymerase (5U/ µl) (BioLabs, UK)	0.2 µl
6	Template DNA (20ng/µl)	2.0 µl
7	Sterile distilled water	18.8 µl
	Total volume	25.0 µl

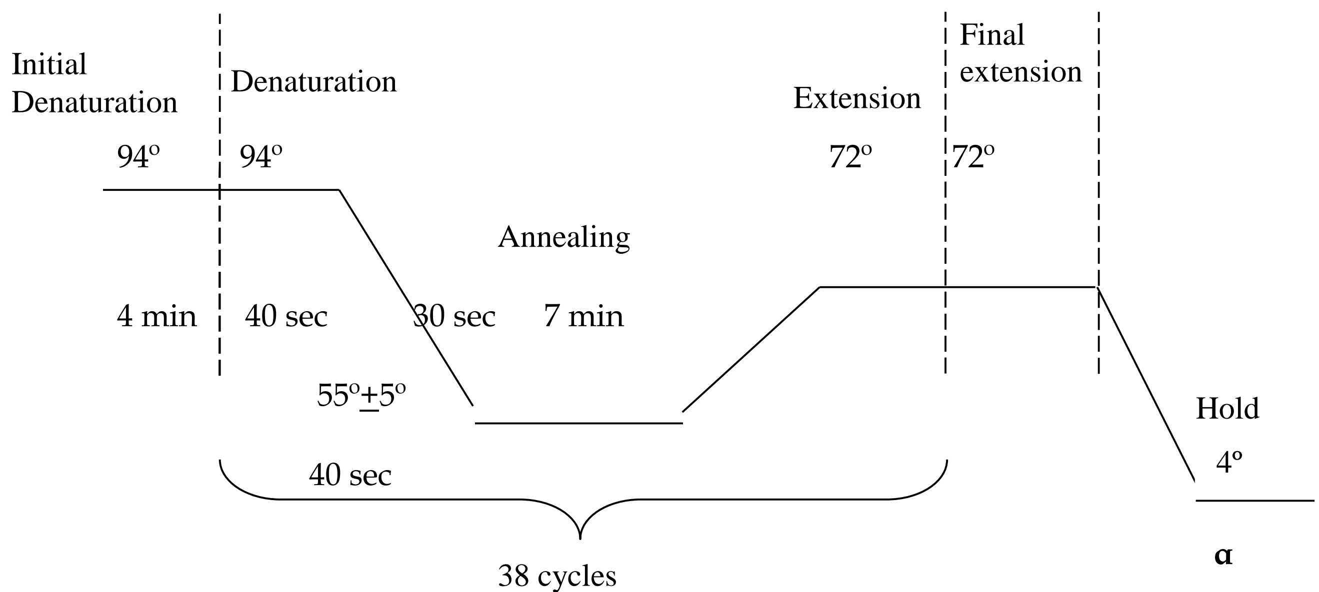


Figure 3.1: PCR reaction conditions for SSR primers

3.9.2.2 Electrophoresis of amplified products

The amplified products of SSR were analyzed electrophoretically using 2.5% agarose gel.

3.9.2.3 Chemicals used:

- Agarose (Invitrogen, Brazil)

- 5 X Tris Borate EDTA (TBE) buffer pH 8.0
- Ethidium bromide (10 mg/ml)
- 100 bp DNA ladder (Fermentas, USA)

Agarose gel of 2.5 % concentration was prepared in 0.5X TBE (2.5 g agarose in 100 ml 0.5X TBE and 4µl Ethidium bromide 10mg/ml. PCR amplified products (9 µl and 1 µl 6X loading dye) were loaded into the wells. The molecular weight marker, 100bp ladders were used for band sizing. The electrophoresis was conducted at a constant voltage of 80 V to separate the amplified bands. The separated bands were visualized under UV transilluminator (GeNie, Bangalore, India) and photographed using gel documentation system (SYNGENE, USA).

3.10 Data analysis

Clear and distinct bands amplified by RAPD and SSR primers were scored for the presence and absence of the corresponding band among the genotypes. The scores 1 and 0 indicates the presence or absence of bands respectively. The softwares used for the analysis of the scored data were NTSYSpc version 2.02 (Rholf 1994). The molecular weight of the PCR products were estimated by Alpha EaseFC4.0.0 software (Alpha Innotech Corporation, USA) for each primer to analyze alleles range.

3.10.1 Genetic similarity and cluster analysis

Coefficients of similarity were calculated by using Jaccard's similarity coefficient by SIMQUAL function and cluster analysis was performed by agglomerative technique using the UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) method by SAHN clustering function

of NTSYSpc. Relationships among the pigeonpea genotypes were expressed in the form of dendrograms and genetic similarity matrix. .

3.10.2 Cophenetic correlation and Mantel test

The cophenetic correlation analysis was carried out using the COPH function of NTSYSpc. In this method dendrogram and similarity matrix were correlated to find the goodness of fit of the dendrogram constructed based on similarity coefficients. The Mantel matrix correspondence test was carried out using the MXCOMP function in the NTSYSpc version 2.20N.

3.10.3 Principal Component Analysis (PCA)

Principal component analysis was carried out using the EIGEN module of NTSYSpc version 2.20N. The results were graphically expressed in the form of 2D and 3D plots generated by graphics module after the calculation of EIGEN values. First three Eigen values which showed maximum variation were extracted as principal components and 2D and 3D plots were generated on that basis.

3.10.4 Calculation of parameters of genetic variability

Various components were calculated which included, No. of monomorphic and polymorphic loci, Polymorphism Information Content (PIC), Effective Multiplex ratio (EMR), Marker Index (MI), Polymorphism % etc. Polymorphism Information content (PIC) was calculated according to formula described by Bootstein *et al.*, (1980) and (Anderson *et al.*,1993).

$$PIC = 1 - \sum_{i=1}^{k} Pi^2$$

Where k is total number of alleles detected for a given marker locus and Pi is the frequency of the ith allele in the set of genotypes investigated.

Marker Index is calculated according to Powell *et al.*, (1996).

Marker Index (MI) = EMR X DI (av) p

Where Effective Multiplex Ratio (EMR) is the fraction of polymorphic loci and the number of polymorphic loci for an individual assay.

EMR = np(np/n) where n=np+nnp, np=polymorphic loci, nnp=non-polymorphic loci and DI(av)p=average Diversity Index of Polymorphic loci = ∑DIn/np (DI=PIC), ∑DIn = ∑PIC.

All the above mentioned variables were calculated individually for RAPD and SSR markers to identified genotypes of pigeonpea which are resistant/susceptible to fusarium wilt.

3.10.5 A summary of the statistics used in analysis of SSR markers

The analysis was performed using GenALEx 6.5 software and calculated manually in Microsoft Excel 2007 (Peakall and Smouse, 2006).

Allele Frequency (Codominant Data)

$$FreqAllele_x = \frac{2Nxx+Nxy}{2N}$$

Calculated for a single locus. Determined for each allele. Nxx = # of XX homozygous individuals, and Nxy = # of XY heterozygous individuals, where Y can be any other allele. N = the number of samples. Can also be determined simply by direct count of the proportion of different alleles (Nei M, 1987).

Observed Heterozygosity (Ho) (Codominant Data)

$$H_o = \frac{No_of_Hets}{N}$$

This is calculated on a per locus basis. GenAlEx also provides the arithmetic mean across loci. Where the number of heterozygotes is determined by direct count and N = the number of samples (Nei M, 1987).

Expected Heterozygosity (He) or Genetic Diversity (Codominant Data)

$$He = 1 - \sum pi2$$

Calculated on a single locus basis. GenAlEx also provides the arithmetic mean across loci. Here, pi is the frequency of the ith allele.

CHAPTER IV
RESULTS AND DISCUSSION

Okra (*Abelmoschus esculentus* L.) is an economically important vegetable crop is grown in tropical and subtropical parts of the world. Okra immature fruits consumed as a vegetables, can be used as a salad, soups. Okra is the powerhouse of vegetable nutrients, having soluble fiber and pectins which help to reducing the risk of heart disease. The field experiment was conducted at Main vegetable research station, Anand agricultural university, Anand during Kharif 2015 to study "Characterization of Okra (*Abelmoschus esculentus* L) Genotypes through Biochemical and Molecular markers". The results are presented below.

4.1 Morphological Attributes

4.1.1 Plant height at harvest (cm)

Plant height is a central part of plant ecological strategy. It is strongly correlated with life span, seed mass and time to maturity and is a major determinant of a species ability to compete for light.

The present investigation clearly indicated that okra plant height was ranging from 61.26 – 127.06 (cm) (Table 4.1 & Fig.4.1). Significantly maximum plant height was registered for Red okra (127.06 cm), which was at par with AOL 09-02 (123.00 cm).The non significant differences were observed for plant height among AOL 13-94(61.26 cm), AOL 13-90(61.33 cm), and AOL 14-29(64.31 cm).

Table 4.1:- Morphological attributes of different genotypes of okra.

Genotypes	Plant height (cm)	Fruit length (cm)	Fruit girth(cm)	Fruit weight (g)	Fruit volume (cm^3)	Fruit density (g/cm^3)
AOL 12-52	100.31	12.46	5.50	11.90	9.70	1.22
AOL 14-32	82.26	11.13	6.16	9.59	7.43	1.29
AOL 13-94	61.26	7.56	6.93	6.66	6.00	1.11
AOL 09-02	123.00	9.26	5.90	11.96	9.46	1.26
AOL 14-29	64.31	10.70	6.33	11.96	9.90	1.21
JOL 11-12	93.00	8.03	5.26	7.96	5.73	1.39
AOL 13-141	71.37	11.66	6.43	10.82	7.96	1.36
JDNO 11-11	100.43	10.76	5.63	9.83	8.46	1.17
Kashi Kranti	94.33	11.96	6.56	10.53	8.90	1.18
JDNO 11-12	95.33	11.80	5.90	13.68	9.93	1.37
AOL 13-133	96.00	10.70	6.56	12.24	7.96	1.56
JOL 09-05	85.33	10.93	5.80	12.16	9.63	1.26
AOL 14-11	82.67	11.76	7.50	13.09	9.83	1.33
JOL 13-05	98.00	11.83	5.73	11.60	9.66	1.20
GP OK 296	82.33	11.16	6.10	10.92	8.13	1.34
GP OK 213	87.20	2.03	2.38	2.13	2.00	1.06
GP OK 292	73.49	12.73	7.83	17.30	10.26	1.69
GAO-5	97.96	10.90	6.80	6.56	5.76	1.14
Red Okra	127.06	2.76	5.66	3.68	3.33	1.28
Pusa Sawani	98.36	8.73	5.46	9.09	6.63	1.37
Parbhani Kranti	86.33	12.76	5.13	12.53	9.83	1.27
AOL 13-73	81.33	7.80	5.16	8.66	6.93	1.25
AOL 12-55	73.10	6.80	4.73	5.93	4.63	1.28
AOL 13-88	79.33	9.33	5.36	9.59	8.60	1.12
AOL 13-112	95.33	9.83	6.73	13.82	10.20	1.35
AOL 14-08	89.46	10.46	7.03	14.58	10.46	1.39
AOL 13-90	61.33	7.03	5.10	6.93	5.96	1.18
AOL 12-59	96.00	9.23	6.50	11.03	8.56	1.29
JOL 13-07	100.00	9.70	5.60	8.06	6.53	1.23
AOL 13-144	95.66	10.63	6.86	13.75	9.70	1.42
S.Em.±	2.52	0.35	0.24	0.50	0.39	0.22
C.D.@5%	7.29	0.986	0.673	1.42	1.12	0.610
CV%	4.89	6.01	6.83	8.45	8.61	8.02

On the basis of this study, it can be concluded that the maximum and minimum plant height was observed for Red okra and AOL 13-94, respectively.

Rajkumar and his coworkers (2014) studied on genetic parameters in okra (*Abelmoschus esculentus* (L.) with thirty genotypes. The result indicated that heritability estimates were observed high for plant height at 120 DAS (day after sowing) than 60 DAS .

Rahman and his colleagues (2012) have reported maximum and minimum okra plant height in cv. Puja (96.83 cm) and cv. Anmol (63.40 cm), respectively.

Muhammad and his coworkers (2012) found that the plant height of various okra genotypes was ranging from 101 – 114 cm. The growth of okra was more vigorous in the open field. The highest average plant height (131.2 cm) of okra variety BARI Dherosh-1 was recorded by (Hasan *et. al.*, 2012).

4.1.2 Fruit length (cm)

The okra fruit length was measured and the data are presented in Table 4.1 and Fig 4.2. Significantly higher fruit length was recorded for Parbhani Kranti (12.76 cm), which was at par with GP OK-292 (12.73 cm), the significantly lower fruit length was observed for GP OK-213 (2.03 cm). Non significant differences were recorded among AOL 14-29, AOL 13-133, GAO-5, AOL 14-08 and JOL 09-05.

On the basis of the study, it can be concluded that the maximum and minimum fruit length was observed for Parbhani kranti and GP OK-213 respectively.

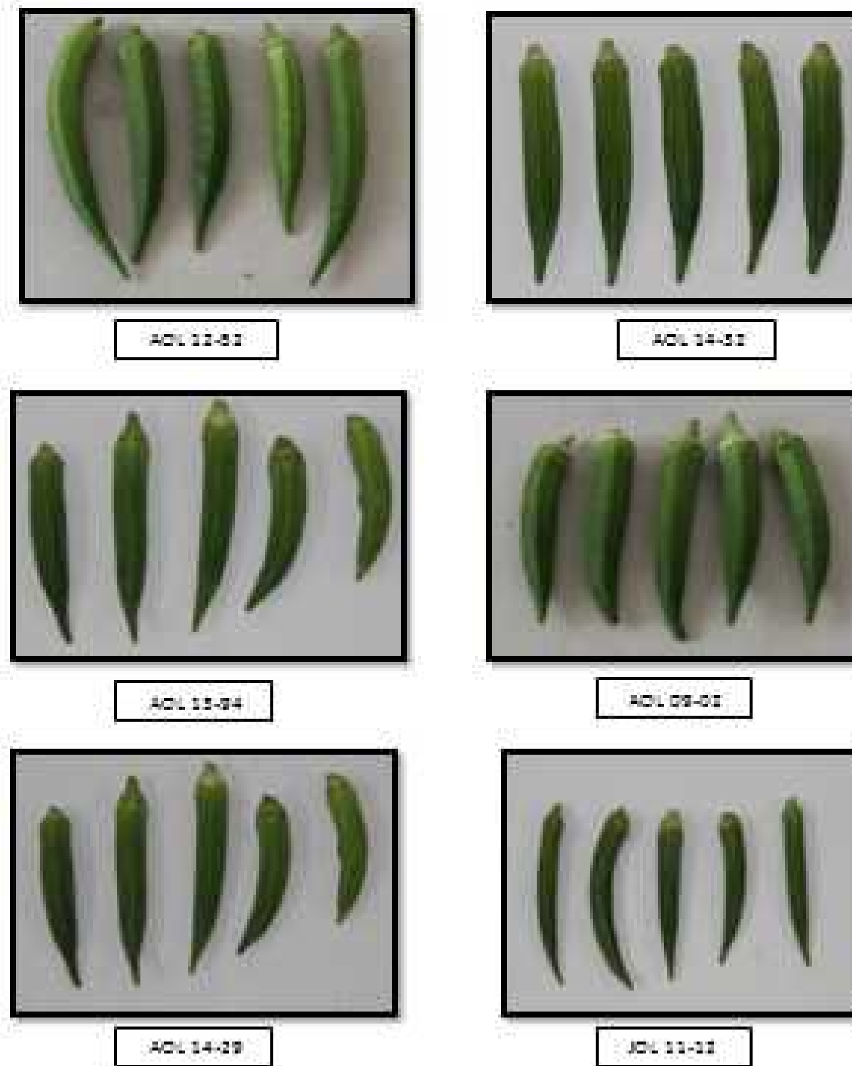


Plate 4.1.: Phenotypic variation in thirty genotypes

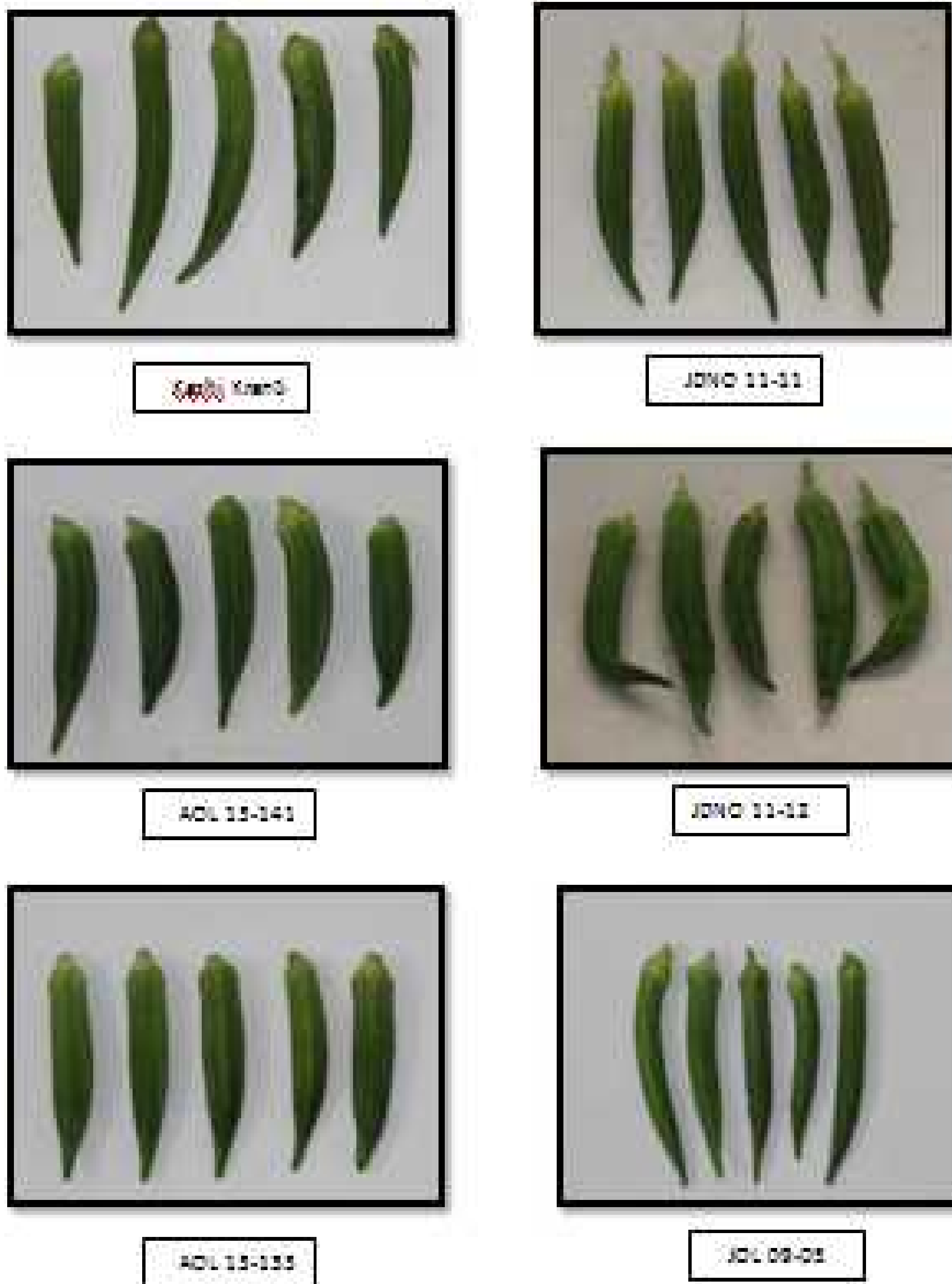
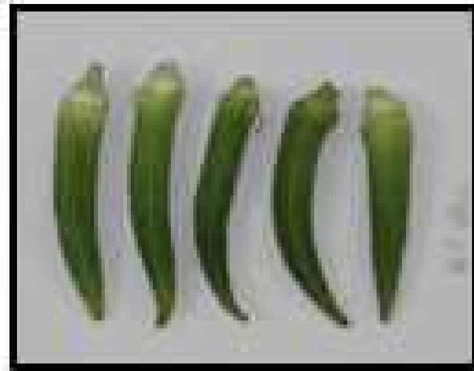


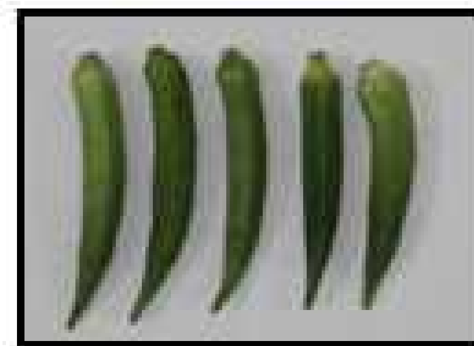
Plate 4.1.- Phenotypic variation in thirty genotypes

AOL 14-11



JOL 13-05

GP OK-296



GP OK-292

GAO-5

Pusa Sawani

Plate 4.1.: Phenotypic variation in thirty genotypes

Parbhani Kranti

AOL 13-73

AOL 12-55

Red Okra

AOL 13-88

AOL 13-112

Plate 4.1: Phenotypic variation in thirty genotypes

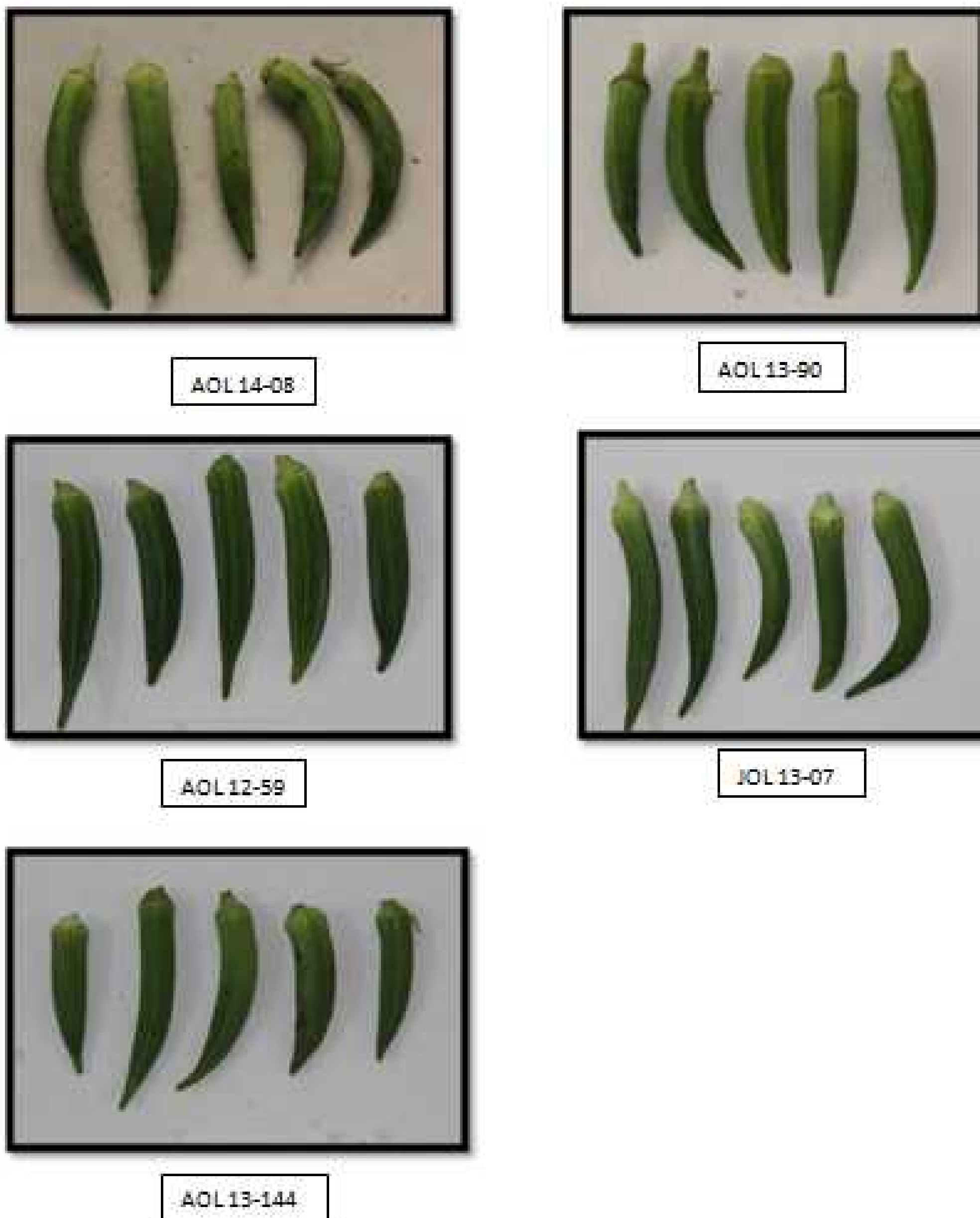


Plate 4.1.: Phenotypic variation in thirty genotypes

Kabir and Pillu (2011) found that the okra fruit length was maximum 11.83 cm in Shagun followed by 11.75cm in Arka Anamika and 11.16 cm in Sinnova.

4.1.3 Fruit girth (cm)

The fruit girth of okra ranged between 2.38 – 7.83 cm (Table 4.1 & Fig.4.3).The maximum fruit girth recorded for GP OK-292, which was followed by AOL 14-11 (7.50 cm) and AOL 14-08 (7.03 cm).The minimum fruit girth was observed for GP OK-213 (2.38cm).

On the basis of the study, it can be concluded that the maximum and minimum fruit length was observed for GP OK-292 and GP OK-213 respectively.

Tapankumar *et al.,* (2009) studied the different okra cultivars and found that higher fruit girth was 1.69 cm in Makhmali, while lower fruit girth was 1.51 cm in INDO-321.

4.1.4 Fruit weight (gm)

The highest fruit weight was recorded for GP OK 292 (17.30 gm) followed by AOL 14-08 (14.58 gm).Minimum fruit weight was (Table 4.1 & Fig. 4.4) recorded for GP OK 213 (2.13 gm), and it was statistically at par with Red okra (3.68 gm).

On the basis of the study, it can be concluded that the maximum and minimum fruit length was observed for GP OK-292 and GP OK-213, respectively.

Kabir and Pillu (2011) studied fruit weight of okra at different stages. In this study maximum fruit weight was found with Sinnova (12.77gm) followed by in Shagun (12.39 gm) and Arka Anamika (10.23gm).

4.1.5 Fruit volume (cm^3)

The fruit volume of okra determined by liquid displacement method and data are presented in Table 4.1 & Fig.4.5. Significantly higher fruit volume was registered for AOL 14-08 (10.46 cm^3), which was statistically at par with GP OK-292 (10.26 cm^3), AOL 13-112 (10.20 cm^3), JDNO 11-12 (9.93 cm^3), AOL14-29 (9.90 cm^3) and AOL-14-11 (9.83 cm^3).Whereas, significantly lower fruit volume was recorded for GP OK-213 (2.00 cm^3).

On the basis of this study, it can be concluded that the maximum and minimum fruit volume was observed for AOL 14-08 and GP OK-213 respectively.

Ikrang (2014) observed that average value of volumes of three fruits of okra was 13.28 cm^3.

4.1.6 Fruit density (g/cm^3)

Density is the ratio of weight to volume. Fruit density was calculated and presented in Table 4.1, & Fig. 4.6. The maximum and minimum fruit density was recorded for GP OK-292 (1.69 g/cm^3), and GP OK-213 (1.06g/cm^3), respectively.

On the basis of this study, it can be concluded that the maximum and minimum fruit length was registrated for GP OK-292 and GP OK-213, respectively.

Ikrang (2014) reported that average value of fruit density in the three fruits of okra was 1.04 g/cm^3.

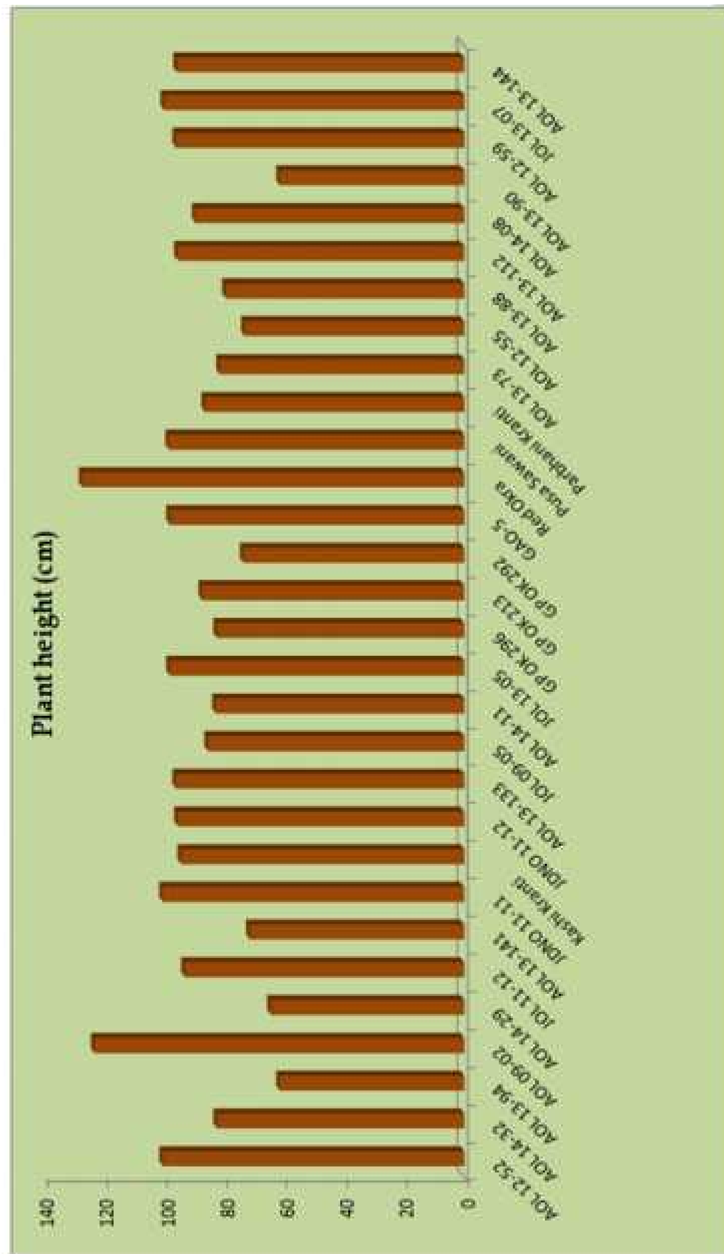


Fig. 4.1:- Plant height of different genotype of okra

Table 4.2: Fruit length of different genotypes of okra.

Fruit girth(cm)

Fig. 4.3:- Fruit girth of different genotype of okra

Fig. 4.4:- Fruit weight of different genotype of okra.

Fig. 4.5:- Fruit volume of different genotype of okra.

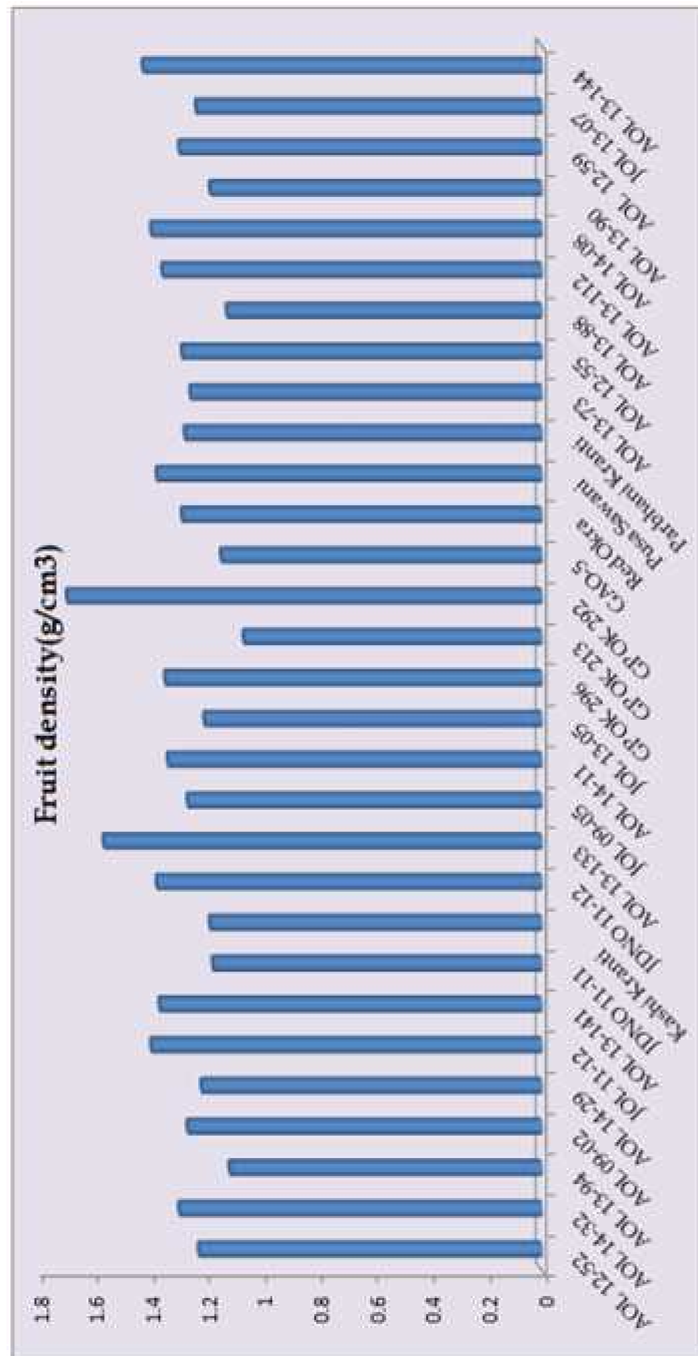


Fig. 4.6:- Fruit density of different genotype of okra

4.2 Phytochemical Attributes

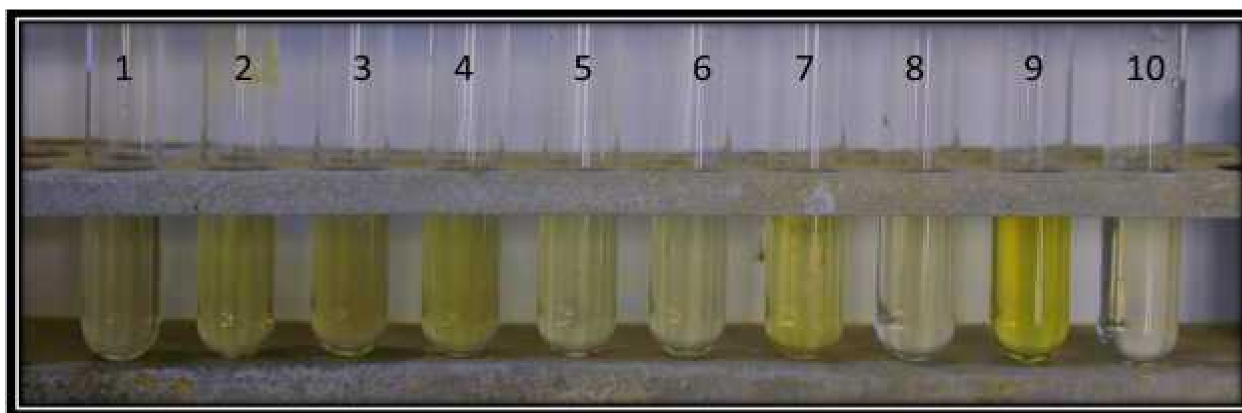
During the present investigation total of five parameters/attributes were determined for thirty (30) genotypes of okra seed. Brief description concerning the studied characters is furnished below and it also represented in Table 4.2.

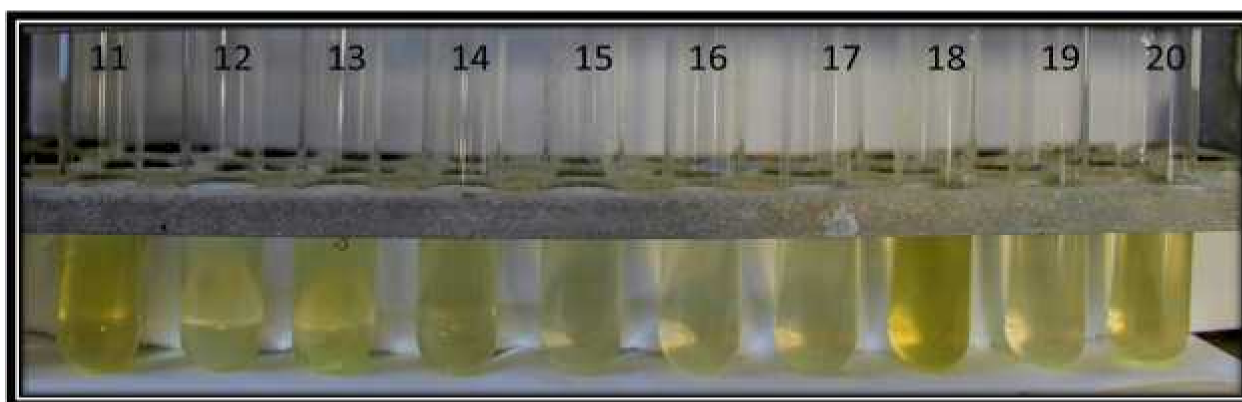
4.2.1 Flavanoids

The higher concentration of flavanoids was observed in Kashi kranti, AOL 13-73, GAO-5,AOL 14-08 , AOL 13-90, and JOL 13-07.The moderate concentration of flavanoids present in AOL 14-32 ,AOL 13-94,JOL 11-12, AOL 14-11, Pusa sawani, AOL 12-55, AOL 13-88, AOL 13-112, AOL 12-59 and AOL 13-144. The lower concentration of flavanoids present in AOL 12-52, AOL 09-02, AOL 14-29, JOL 09-05, JOL 13-05, GP OK-296, GP OK-213, GP OK-292, Red okra, Parbhani kranti and AOL 13-73. Flavanoids are absent in JOL 11-12, JDNO 11-11.

4.2.2 Glycosides

The higher concentration of glycosides present in AOL 14-32 , JOL 11-12, AOL 14-11, moderate concentration of glycosides present in AOL 09-02, Kashi kranti, JDNO 11-12, AOL 13-133, lower concentration of glycosides present in AOL 12-52, AOL 13-94, AOL 13-141, JDNO 11-11, JOL 09-05, JOL 13-05, GP OK -296, GP OK-292, GAO-5, Red okra, Parbhani kranti, AOL 13-73, AOL 12-55, AOL 13-88, AOL 13-112 , AOL 13-90, AOL 12-59, JOL 13-07 and AOL 13-144. Glycosides are absent in AOL 14-29, GP OK -213, Pusa sawani and AOL 14-08.





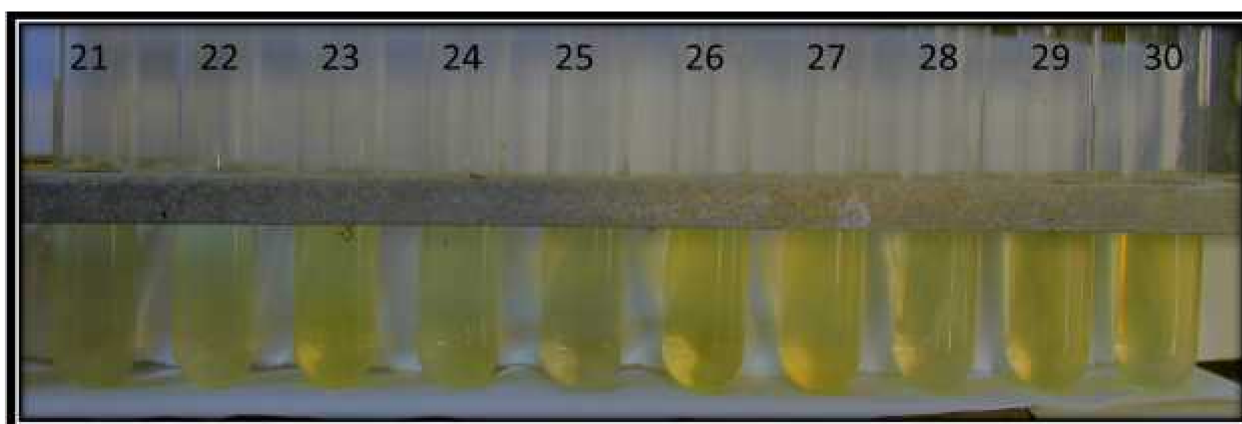
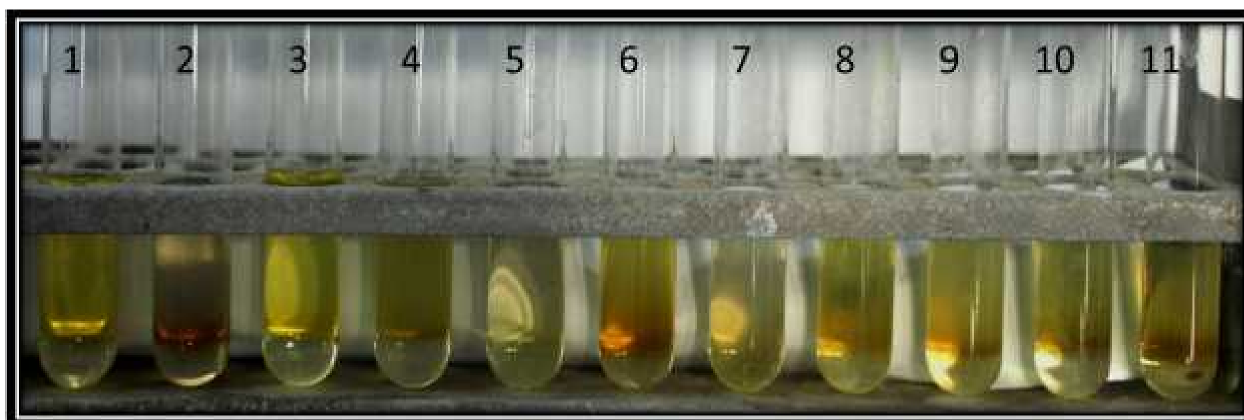
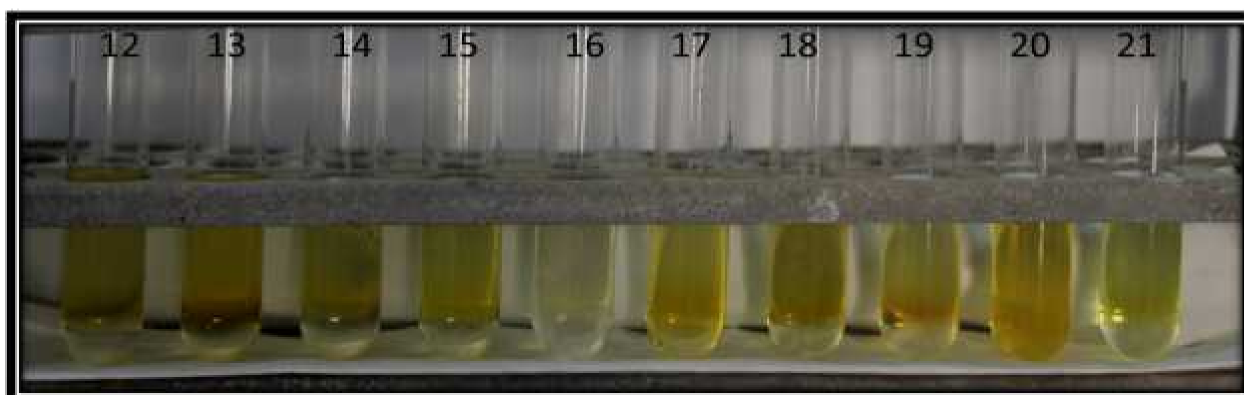


Plate 4.2: Phytochemical screening of flavanoids from okra seed

(1) AOL 12-52 **(2)** AOL 14-32 **(3)** AOL 13-94 **(4)** AOL 09-02 **(5)** AOL 14-29 **(6)** JOL 11-12 **(7)** AOL 13-141 **(8)** JDNO 11-1 **(9)** Kashi kranti **(10)** JDNO 11-12 **(11)** AOL 13-133 **(12)** JOL 09-05 **(13)** AOL 14-11 **(14)** JOL 13-05 **(15)** GP OK-296 **(16)** GP OK-213 **(17)** GP OK -292 **(18)** GAO-5 **(19)** Red okra **(20)** Pusa sawani **(21)** Parbhani karnti **(22)** AOL 13-73 **(23)** AOL 12-55 **(24)** AOL 13-88 **(25)** AOL 13-112 **(26)** AOL 14-08 **(27)** AOL 13-90 **(28)** AOL 12-59 **(29)** JOL 13-07 **(30)** AOL 13-144





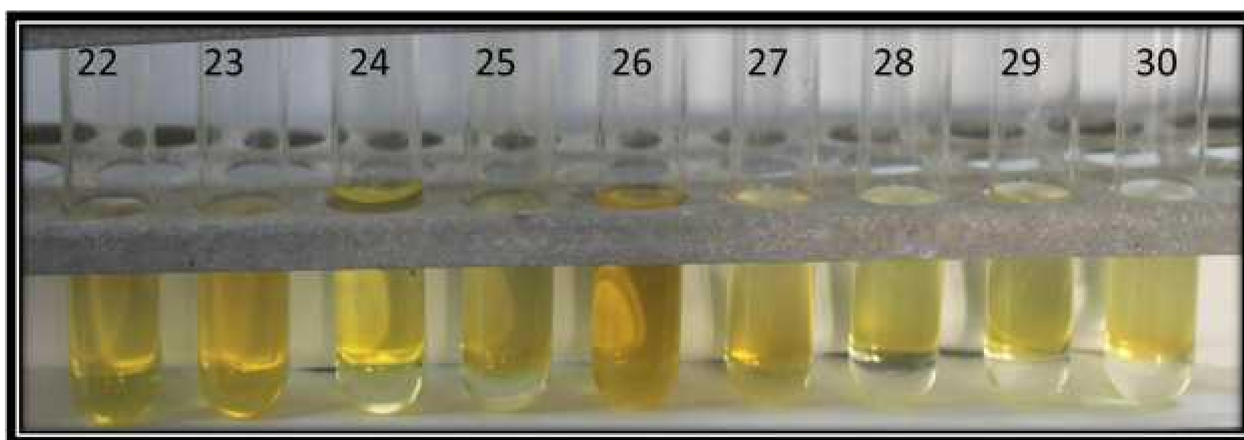


Plate 4.3: Phytochemical screening of glycosides from okra seed

(1) AOL 12-52 **(2)** AOL 14-32 **(3)** AOL 13-94 **(4)** AOL 09-02 **(5)** AOL 14-29 **(6)** JOL 11-12 **(7)** AOL 13-141**(8)** JDNO 11-1 **(9)** Kashi kranti **(10)** JDNO 11-12 **(11)** AOL 13-133 **(12)** JOL 09-05 **(13)** AOL 14-11 **(14)** JOL 13-05 **(15)** GP OK-296 **(16)** GP OK-213 **(17)** GP OK -292 **(18)** GAO-5 **(19)** Red okra **(20)** Pusa sawani **(21)** Parbhani karnti **(22)** AOL 13-73 **(23)** AOL 12-55 **(24)** AOL 13-88 **(25)** AOL 13-112 **(26)** AOL 14-08 **(27)** AOL 13-90 **(28)** AOL 12-59 **(29)** JOL 13-07 **(30)** AOL 13-144

Table 4.2: Phytochemical Attributes of okra seed

Genotypes	Tannins	Alkaloids	Flavanoids	Glycosides	Terpenoids
AOL 12-52	-	-	+	+	-
AOL 14-32	-	-	++	+++	+++
AOL 13-94	-	-	++	+	-
AOL 09-02	-	-	+	++	+++
AOL 14-29	-	-	+	-	-
JOL 11-12	-	-	-	+++	+++
AOL 13-141	-	-	++	+	-
JDNO 11-11	-	-	-	+	+
Kashi kranti	-	-	+++	++	++
JDNO 11-12	-	-	-	++	+++
AOL 13-133	-	-	+++	++	++
JOL 09-05	-	-	+	+	++
AOL 14-11	-	-	++	+++	++
JOL 13-05	-	-	+	+	+
GP OK 296	-	-	+	+	+
GP OK 213	-	-	+	-	-
GP OK 292	-	-	+	+	-
GAO-5	-	-	+++	+	++
Red okra	-	-	+	+	+++
Pusa sawani	-	-	++	-	++
Parbhani kranti	-	-	+	+	-
AOL 13-73	-	-	+	+	+
AOL 12-55	-	-	++	+	+
AOL 13-88	-	-	++	+	++
AOL 13-112	-	-	++	+	+++
AOL 14-08	-	-	+++	-	++
AOL 13-90	-	-	+++	+	+
AOL 12-59	-	-	++	+	++
JOL 13-07	-	-	+++	+	++
AOL 13-144	-	-	++	+	++

4.2.3 Terpenoids

The higher concentration of terpenoids present in AOL 14-32, AOL 09-02, JOL 11-12, JDNO 11-12, Red okra, AOL13-112.The moderate concentration of terpenoids present in Kashi kranti , AOL 13-133, JOL 09-05, AOL 14-11, GAO-5, Pusa sawani, AOL 13-88, AOL 14-08, AOL 12-59, JOL 13-07 and AOL 13-144, while the lower concentration of terpenoids observed in JDNO 11-11, JOL 13-05,GP OK-296, GP OK-213, AOL 13-73, AOL 12-55 and AOL 13-90. Terpenoids are absent in AOL 12-52, AOL 13-94, AOL 14-29, AOL 13-141,GP OK-213, GP OK-292 and Parbhani kranti.

4.2.4 Tannins

There are thirty okra genotypes were studied for tannins. In all the okra genotypes tannins are absent, it showed that tannins are absent in okra seed.

4.2.5 Alkaloids

There are thirty okra genotypes were studied for alkaloids. In all the genotypes alkaloids are absent.

Wesley and his coworkers (2013) studied the phytochemical screening of various extract from *Abutilon hirtum* in which Flavanoids, terpenoids, glycosides, are present and tannins, alkaloids are absent from water extract .

Overall it can be concluded from the present investigation that the higher concentration of flavanoids were determined in, Kashi kranti, AOL 13-73, GAO-5, AOL 14-08, AOL 13-90, and JOL 13-07 were glycosides are present in higher concentration *viz*., AOL 09-02, Kashi kranti, JDNO 11-12 and AOL 13-133 ,while terpenoids are present in *viz*., AOL 14-32, AOL 09-02, JOL 11-12, JDNO 11-12, Red

okra and AOL 13-112. Only flavanoids are absent in two genotypes *viz.,* AOL 14-29 and GP OK-213. The alkaloids and tannins are absent in all thirty genotypes of okra seed.

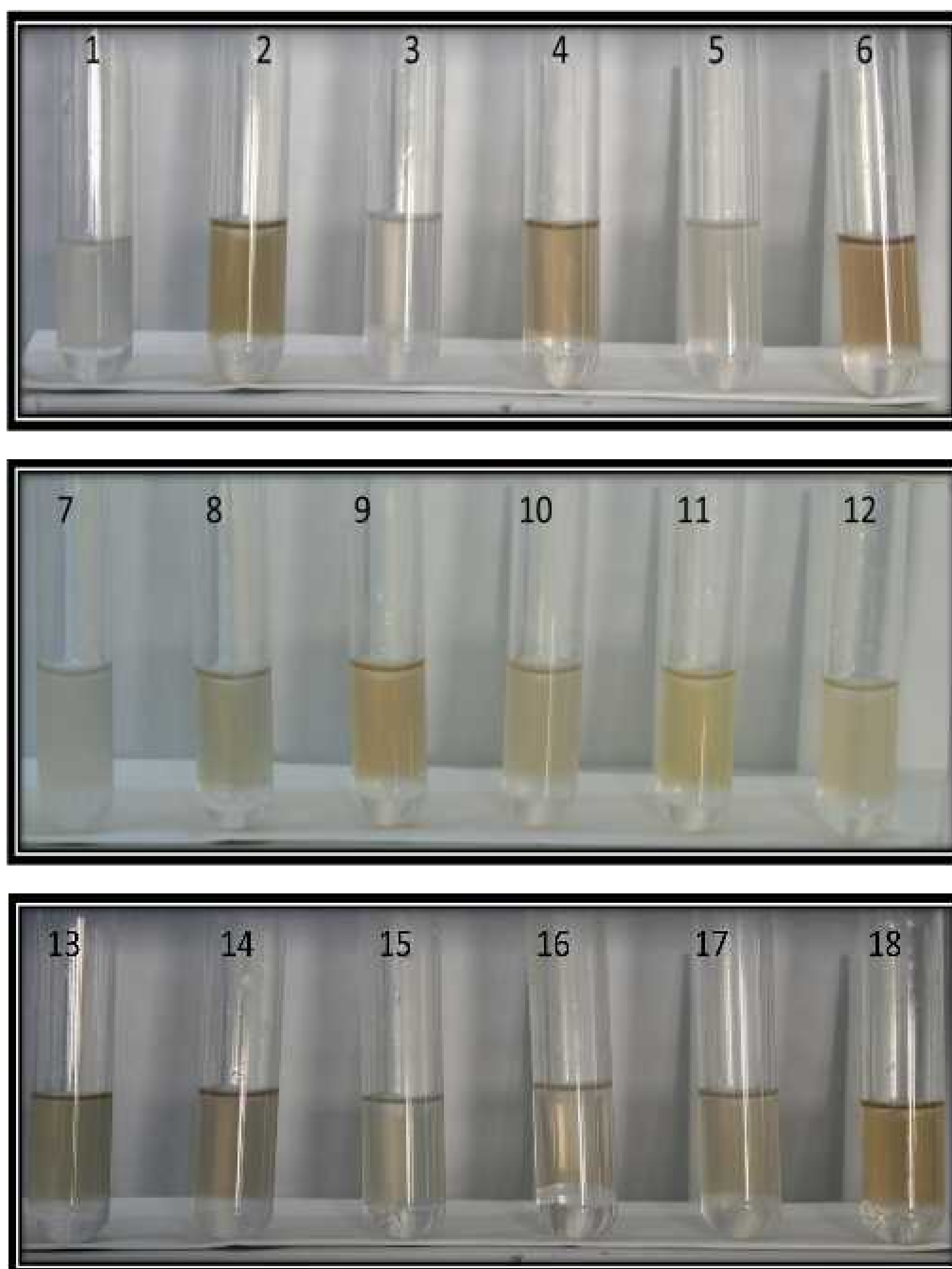


Plate 4.4$_a$: Phytochemical screening of terpenoids from okra seed

(1) AOL 12-52 **(2)** AOL 14-32 **(3)** AOL 13-94 **(4)** AOL 09-02 **(5)** AOL 14-29 **(6)** JOL 11-12 **(7)** AOL 13-141 **(8)** JDNO 11-1 **(9)** Kashi kranti **(10)** JDNO 11-12 **(11)** AOL 13-133 **(12)** JOL 09-05 **(13)** AOL 14-11 **(14)** JOL 13-05 **(15)** GP OK-296 **(16)** GP OK-213 **(17)** GP OK -292 **(18)** GAO-5

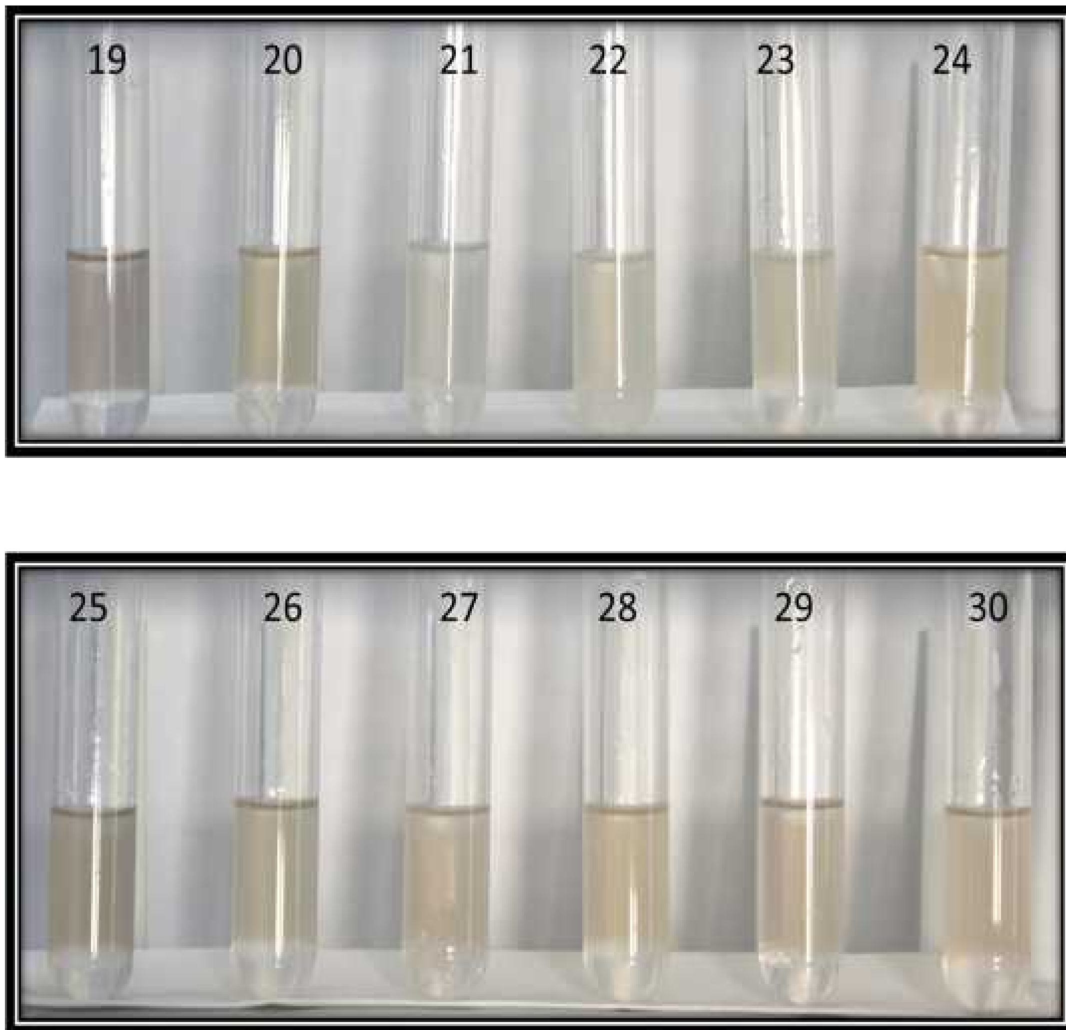


Plate 4.4$_b$: Phytochemical screening of glycosides from okra seed

(19) Red okra **(20)** Pusa sawani **(21)** Parbhani karnti **(22)** AOL 13-73 **(23)** AOL 12-55 **(24)** AOL 13-88 **(25)** AOL 13-112 **(26)** AOL 14-08 **(27)** AOL 13-90 **(28)** AOL 12-59 **(29)** JOL 13-07 **(30)** AOL 13-144 .

4.3 Biochemical Attributes

4.3.1 Moisture

Moisture deficiency brings about low quality and quantity of the yield and finally results in plant death.

Moisture content was measured in seed of different okra cultivars and presented in (Table 4.3).Moisture content ranged from 2.38 to 7.02 percent.

Significantly higher moisture content was recorded in JDNO 11-11 (7.02%). Significantly the lowest moisture content was recorded for AOL 14-32 (2.38%), which is at par with AOL 12-52(2.85%), GAO-5(2.65%).

On the basis of this study, it can be concluded that the maximum and minimum moisture content was observed for JDNO11-11 and AOL 14-32 respectively.

Dhruve *et al.* (2015) studied 10 different cultivars of okra, they have found significantly the lowest moisture content was recorded with GAO 5(2.69%), while the higher moisture content was recorded with AOL-13-88 (6.40%) which was at par with AOL 13-90 (6.26%).

4.3.2 True protein

Protein is an important biochemical factor for disease resistance in plant. From seed the maximum protein content was observed in GAO-5 (18.93%) followed by Pusa sawani (18.63%), AOL 09-02 (18.56 %), Parbhani kranti(18.28%) and Red okra (18.20%) while the minimum protein content was observed in AOL 13-141 (13.89%) followed by, AOL 13-88 (14.02 %) ,AOL 13-112 (14.07%) (Table 4.3).

On the basis of this study, it can be concluded that the maximum and minimum true protein was recorded for GAO-5 and AOL13-141 respectively.

Dhruve *et al*, (2015) analyzed the okra genotypes the highest and the lowest protein content was in GAO-5 (18.96%) and in AOL-13-88(14.05%).

4.3.3 Crude protein

It is the average percentage of nitrogen in the food proteins and that may contain an appreciable error if the nitrogen is derived from non protein material or

from a protein of unusual composition. The maximum protein content (Table 4.3) was observed in GAO-5 (22.01 %) followed by Pusa sawani (21.33 %), Kashi kranti (21.28 %) and AOL 09-02 (21.02%), while the minimum protein content was observed in AOL 13-112 (15.87 %) followed by AOL 13-88 (16.03 %).

On the basis of this study, it can be concluded that the maximum and minimum crude protein was observed for GAO-5 and AOL 13-112, respectively.

Gemede and his coworkers (2015) investigated eight okra accessions, and they found that the crude protein range of okra in between 10.25 - 26.16%.

4.3.4 Total lipids

Okra seed oil is a rich source of unsaturated fatty acids. Okra seed oil is also a rich source of linoleic acid, a polyunsaturated fatty acid essential for human nutrition. In the present experiment the maximum oil content recorded (Table 4.3) in Kashi kranti (16.01%) followed by JDNO 11-11 (15.50%), AOL 13-133 (14.66%) and the minimum oil content recorded in AOL 14-29 (10.33%) followed by AOL 14-08 (10.50%) , AOL 12-59 (10.66%).

On the basis of this study, it can be concluded that the maximum and minimum crude protein was observed for Kashi kranti and AOL 14-29, respectively.

Dhruve *et al.*, (2015) found that the highest and the lowest oil content were recorded in AOL 10-22 (15.70%) and Red okra (10.94%) respectively.

4.3.5 Free fatty acid %

Fatty acids are found in the triglyceride form, however, during processing the fatty acids may get hydrolyzed into free fatty acid. The higher the acid value found, the higher the level of free fatty acids which translates into decreased oil quality.

In this present investigation measured free fatty acid % and acid value (mg KOH/g) from seeds of different thirty okra genotypes from them the maximum free fatty acid% recorded (table 4.3) in JDNO 11-11 (4.90%) followed by AOL 13-141 (4.89%) ,JOL 13-07(4.44%) ,while minimum free fatty acid% recorded in AOL 14-08 (2.72%) , followed by AOL 12-59 (2.91%) .

On the basis of this study, it can be concluded that the maximum and minimum free fatty acid% was observed for JDNO 11-11 and AOL 14-08 respectively.

Table 4.3: Biochemical constituents in various okra genotypes

Genotypes	Moisture %	Crude protein %	True protein %	Total lipids %	Acid value (mg KOH/g)%	Free fatty acid
AOL 12-52	2.85	19.34	16.67	13.83	7.48	3.76
AOL 14-32	2.38	18.18	16.13	11.28	6.83	3.43
AOL 13-94	3.22	17.48	15.53	11.16	7.18	3.61
AOL 09-02	3.08	21.03	18.56	12.16	7.98	4.01
AOL 14-29	3.35	18.93	16.97	10.33	8.13	4.09
JOL 11-12	3.79	18.25	15.66	14.16	8.72	4.39
AOL 13-141	6.59	16.28	13.89	14.68	9.72	4.89
JDNO 11-11	7.02	20.12	17.67	12.16	9.74	4.90
Kashi kranti	3.78	21.28	17.88	16.01	7.03	3.54
JDNO 11-12	5.31	20.24	17.11	15.50	8.43	4.24
AOL 13-133	4.11	18.87	16.10	14.66	6.13	3.08
JOL 09-05	5.35	19.82	17.44	12.00	7.16	3.60
AOL 14-11	5.70	18.17	15.56	14.33	6.16	3.10
JOL 13-05	5.52	18.55	16.51	11.00	8.56	4.31
GP OK 296	4.98	18.56	16.30	12.16	6.55	3.29
GP OK 213	3.83	18.87	16.67	11.66	6.63	3.33
GP OK 292	3.84	20.43	17.84	12.67	7.44	3.74
GAO-5	2.65	22.01	18.93	14.00	7.41	3.23
Red okra	3.25	20.85	18.55	11.03	8.23	4.14
Pusa sawani	5.08	21.33	18.63	12.66	7.53	3.79
Parbhani kranti	6.58	20.85	18.38	11.83	8.23	4.14
AOL 13-73	3.66	17.02	15.09	11.33	6.82	3.43
AOL 12-55	6.11	17.98	15.73	12.50	7.38	3.71
AOL 13-88	6.04	16.03	14.02	12.66	8.05	4.05
AOL 13-112	4.09	15.87	14.07	11.33	7.15	3.60
AOL 14-08	4.39	18.40	16.47	10.50	5.41	2.72
AOL 13-90	3.00	17.91	15.84	11.56	6.18	3.11
AOL 12-59	5.37	16.83	15.04	10.66	5.78	2.91
JOL 13-07	4.59	19.27	16.51	14.33	8.82	4.44
AOL 13-144	6.70	18.86	16.44	12.83	7.42	3.73
Sem±	0.22	0.24	0.35	0.54	0.11	0.09
C.D .at 5%	0.61	0.68	0.98	1.52	0.32	0.264
C.V.%	8.02	2.74	3.12	7.01	2.54	2.69

4.3.6 Acid value (mg KOH/g)

In this present investigation measured maximum acid value recorded in JDNO 11-11 (9.74 mgKOH/g) followed by AOL 13-141 (9.72 mgKOH/g), while minimum acid value recorded in AOL 14-08 (5.41 mg KOH/g), followed by AOL 12-59 (5.78 mg KOH/g) presented in table 4.3.

On the basis of this both study, it can be indicated that the maximum and minimum acid value was observed for JDNO 11-11 and AOL 14-08 respectively.

Atinafu G. and his coworker (2011), have investigate the total free fatty acid in some commercial total of nine varieties of edible vegetable oils .They found out the results that Niger seed k-7(12.06 mg KOH/g) and rapeseed k-16(10.38 mg KOH/g) have significantly higher acid value than the rest of oil samples *viz*., Cottonseed (7.97 mg KOH/g), olive (7.29 mg KOH/g), rapeseed (7.01 mg KOH/g),sunflower(5.5 mg KOH/g), palm(4.77 mg KOH/g) and had significantly lower acid values than the rest. The minimum acid value was recorded for palm oil.

4.4 Biochemical markers

4.4 .1 Isozyme analysis

The differences in the isozyme banding pattern are due to variations in the amino acid content of the molecule, which in turn is dependent on the sequence of nucleotides in the DNA (Micales *et al*., 1986). Hence the present investigation was carried out to detect the polymorphism among thirty okra genotypes using isozymes (Polyphenol oxidase, Peroxidase and Esterase).

4.4.1 .1 PPO and its Isoenzyme pattern

The Polyphenol oxidase isoenzyme was carried out from fresh seedlings of okra genotypes. The term isozymes proposed as multiple molecular forms of an enzyme, sharing a catalytic activity derived from the tissue (Markert and Moller, 1977) but differ in their physical properties. Electrophoretic pattern of soluble enzymes represent direct manifestation of the genome and can be considered value to determine genetic variation among number of loci. Hence, the present investigation was carried out to detect the polymorphism among the genotypes using isozymes. The results showed total two bands of PPO isoenzyme with Rm value (Table 4.4 & plate 4.5) of 0.34, 0.56. Bands are represent as a intensity which as low, moderate and high ,here in present investigation the high intensity of PPO isozyme present in AOL 13-133, moderate intensity in GP OK-296 ,Red okra, Parbhani kranti, AOL 12-55 and AOL 13-07 ,while low intensity is present in rest of the thirty genotypes at 0.34 Rm value.

4.4.1.2 Peroxidase and its Isoenzyme pattern

The Peroxidase isoenzyme was carried out from fresh seedlings of okra genotypes. The results showed total two bands of peroxidase with Rm value (Table 4.5 & plate 4.6) of 0.29,0.58.Out of which, higher intensity of peroxidase is present in AOL 14-32, JOL 13-05, AOL 13-112 and AOL 13-144, moderate intensity present in AOL 12-52, AOL 09-02, AOL 14-29, JOL11-12, JDNO 11-11, GP OK-296, GAO-5,AOL 13-73,AOL 12-55,AOL 13-88,AOL 13-90 and JOL 13-07.

Table 4.4: Banding Pattern of Polyphenol oxidase.

Sr.No.	Genotype	Rm value	
		0.34	0.56
1	AOL 12-52	+	+
2	AOL 14-32	+	+
3	AOL 13-94	+	+
4	AOL 09-02	+	+
5	AOL 14-29	+	+
6	JOL 11-12	+	+
7	AOL 13-141	+	-
8	JDNO 11-11	+	+
9	Kashi kranti	+	+
10	JDNO 11-12	+	+
11	AOL 13-133	+	+
12	JOL 09-05	+	+++
13	AOL 14-11	+	+
14	JOL 13-05	+	+
15	GP-OK-296	+	++
16	GP-OK-213	+	+
17	GP-OK-292	+	+
18	GAO-5	+	+
19	Red okra	+	++
20	Pusa sawani	+	+
21	Parbhani kranti	+	++
22	AOL 13-73	+	+
23	AOL 12-55	+	++
24	AOL 13-88	+	+
25	AOL 13-112	+	+
26	AOL 14-08	+	+
27	AOL 13-90	+	+
28	AOL 12-59	+	+
29	JOL 13-07	+	++
30	AOL 13-144	+	+

(+: Low Intensity, ++: Moderate intensity, +++: High intensity)

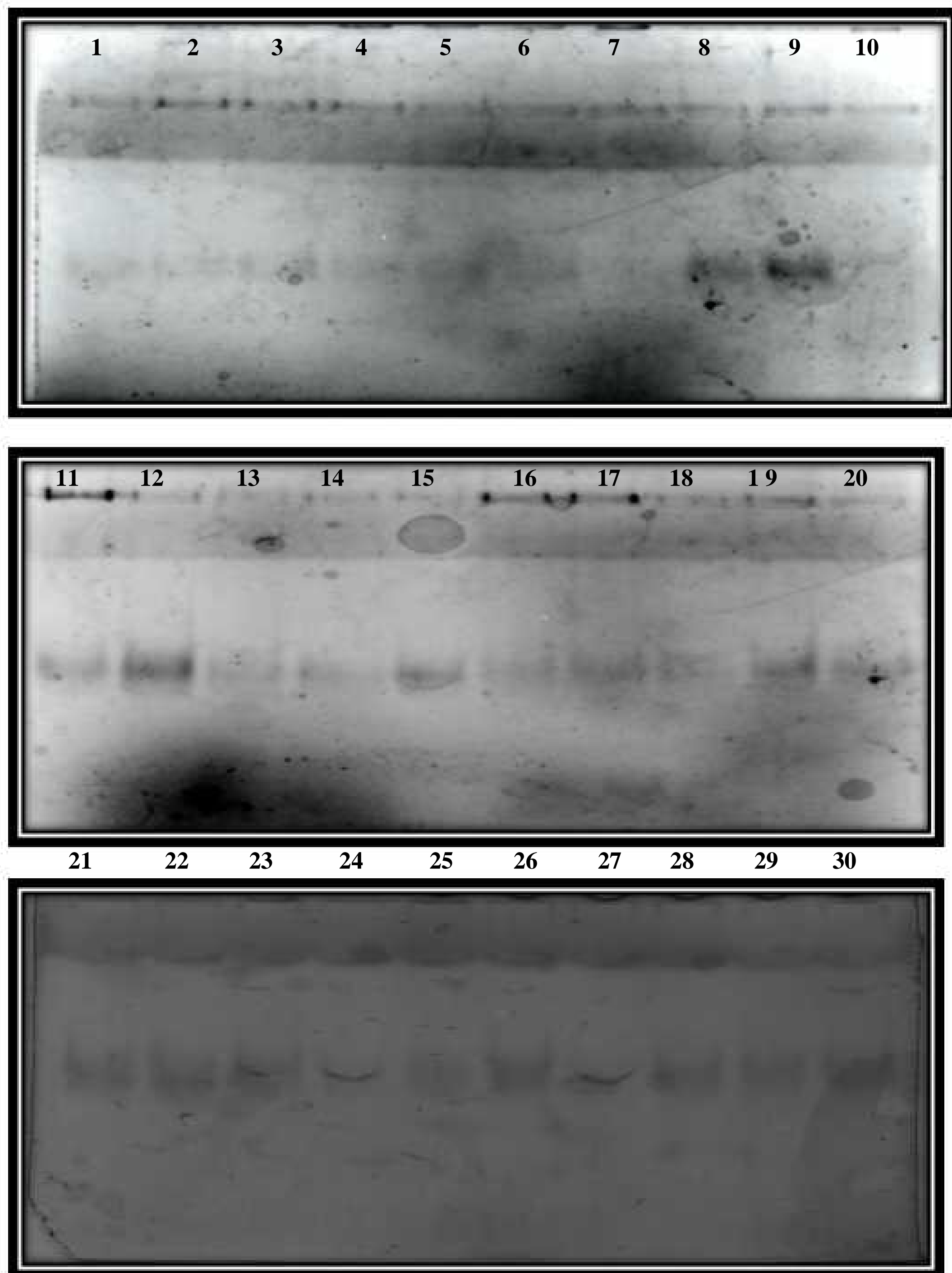


Plate 4.5 : Polyphenol oxidase from okra seedling

(**1**:AOL 12-52, **2**:AOL 14-32, **3**:AOL 13-94, **4**:AOL 09-02, **5**:AOL 14-29, **6**:JOL 11-12, **7**:AOL 13-141, **8**:JDNO 11-11, **9**:Kashi Kranti, **10**:JDNO 11-12, **11**: AOL 13-133, **12**:JOL 09-05, **13**:AOL 14-11, **14**:JOL 13-05, **15**:GP OK-296, **16**:GP OK-213, **17**:GP OK-292, **18**: GAO-5, **19**:Red Okra, **20**:Pusa Sawani, **21**:Parbhani Kranti, **22**:AOL 13-73, **23**:AOL 12-55, **24**:AOL 13-88, **25**: AOL 13-112, **26**:AOL 14-08, **27**:AOL 13-90 , **28**:AOL 12-59, **29**:JOL 13-07 and **30**:AOL 13-144)

Table 4.5: Banding Pattern of Peroxidase

Sr.No	Genotype	Rm value	
		0.29	0.58
1	AOL 12-52	+	++
2	AOL 14-32	+	+++
3	AOL 13-94	-	+
4	AOL 09-02	-	++
5	AOL 14-29	+	++
6	JOL 11-12	-	++
7	AOL 13-141	+	+
8	JDNO 11-11	+	++
9	Kashi kranti	+	++
10	JDNO 11-12	+	++
11	AOL 13-133	+	+
12	JOL 09-05	+	+
13	AOL 14-11	+	+
14	JOL 13-05	+	+++
15	GP-OK-296	-	++
16	GP-OK-213	+	-
17	GP-OK-292	-	-
18	GAO-5	+	++
19	Red okra	-	+++
20	Pusa sawani	+	+
21	Parbhani kranti	+	++
22	AOL 13-73	-	++
23	AOL 12-55	-	++
24	AOL 13-88	-	++
25	AOL 13-112	-	+++
26	AOL 14-08	-	+
27	AOL 13-90	-	++
28	AOL 12-59	-	+
29	JOL 13-07	+	++
30	AOL 13-144	+	+++

(+: low intensity, ++: moderate intensity, +++: high intensity)

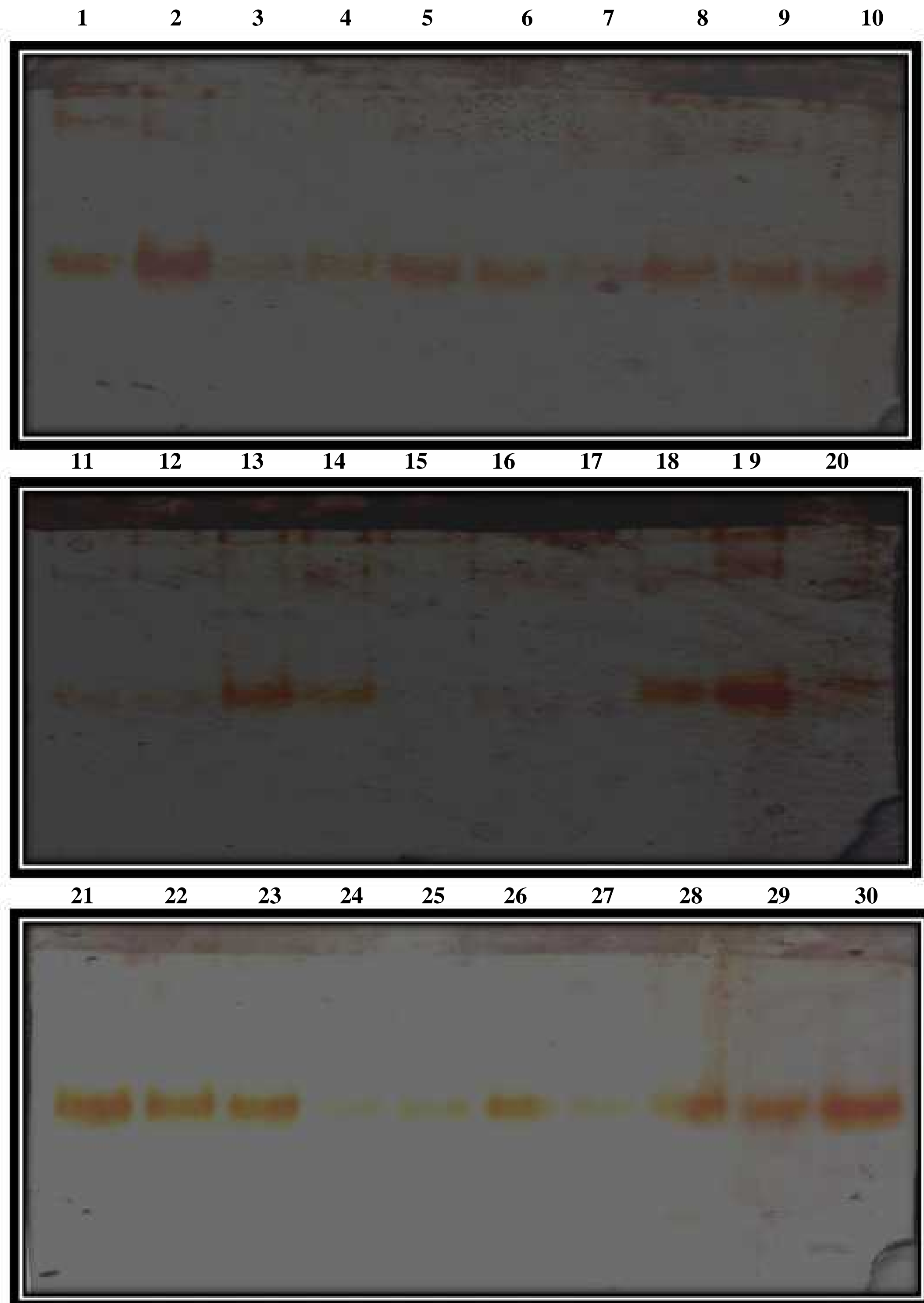


Plate 4.6 : Peroxidasde from okra seedling

(**1**:AOL 12-52, **2**:AOL 14-32, **3**:AOL 13-94, **4**:AOL 09-02, **5**:AOL 14-29, **6**:JOL 11-12, **7**:AOL 13-141, **8**:JDNO 11-11, **9**:Kashi Kranti, **10**:JDNO 11-12, **11**: AOL 13-133, **12**:JOL 09-05, **13**:AOL 14-11, **14**:JOL 13-05, **15**:GP OK-296, **16**:GP OK-213, **17**:GP OK-292, **18**: GAO-5, **19**:Red Okra, **20**:Pusa Sawani, **21**:Parbhani Kranti, **22**:AOL 13-73, **23**:AOL 12-55, **24**:AOL 13-88, **25**: AOL 13-112, **26**:AOL 14-08, **27**:AOL 13-90 , **28**:AOL 12-59, **29**:JOL 13-07 and **30**:AOL 13-144)

4.4.1.3 Esterase and its Isoenzyme pattern

The Esterase isoenzyme was carried out from fresh seedlings of okra genotypes. The results showed total six bands of esterase with Rm value (Table 4.6 & plate 4.7) of 0.25, 0.27, 0.53, 0.56, 0.60 and 0.65. The bands at Rm value 0.60 and 0.65 are about in Pusa sawani and Parbhani kranti, while single band is present in all the genotypes at 0.25, 0.27, 0.53 and 0.56 Rm value.

Table 4.6: Banding Pattern of Esterase

Sr.No	Genotype	Rm value					
		0.25	0.27	0.53	0.56	0.60	0.65
1	AOL 12-52	+	+	+	+	+	+
2	AOL 14-32	+	+	+	+	+	+
3	AOL 13-94	+	+	+	+	+	+
4	AOL 09-02	+	+	+	+	+	+
5	AOL 14-29	+	+	+	+	+	+
6	JOL 11-12	+	+	+	+	+	+
7	AOL 13-141	+	+	+	-	+	+
8	JDNO 11-11	+	+	+	+	+	+
9	Kashi kranti	+	+	+	+	+	+
10	JDNO 11-12	+	+	+	+	+	+
11	AOL 13-133	+	+	+	-	+	+
12	JOL 09-05	+	+	+	+	+	+
13	AOL 14-11	+	+	+	+	+	+
14	JOL 13-05	+	+	+	+	+	+
15	GP-OK-296	+	+	+	+	+	+
16	GP-OK-213	+	+	+	+	+	+
17	GP-OK-292	+	+	+	+	+	+
18	GAO-5	+	+	+	+	+	+
19	Red okra	+	+	+	+	+	+
20	Pusa sawani	+	-	+	+	+	-
21	Parbhani kranti	+	+	+	+	-	+
22	AOL 13-73	+	+	+	+	+	+
23	AOL 12-55	+	+	+	+	+	+
24	AOL 13-88	+	+	+	+	+	+
25	AOL 13-112	+	+	+	+	+	+
26	AOL 14-08	+	+	+	+	+	+
27	AOL 13-90	+	+	+	+	-	+
28	AOL 12-59	+	+	+	-	+	+
29	JOL 13-07	+	+	+	+	+	+
30	AOL 13-144	+	+	+	+	+	+

(+: low intensity, ++: moderate intensity, +++: high intensity)

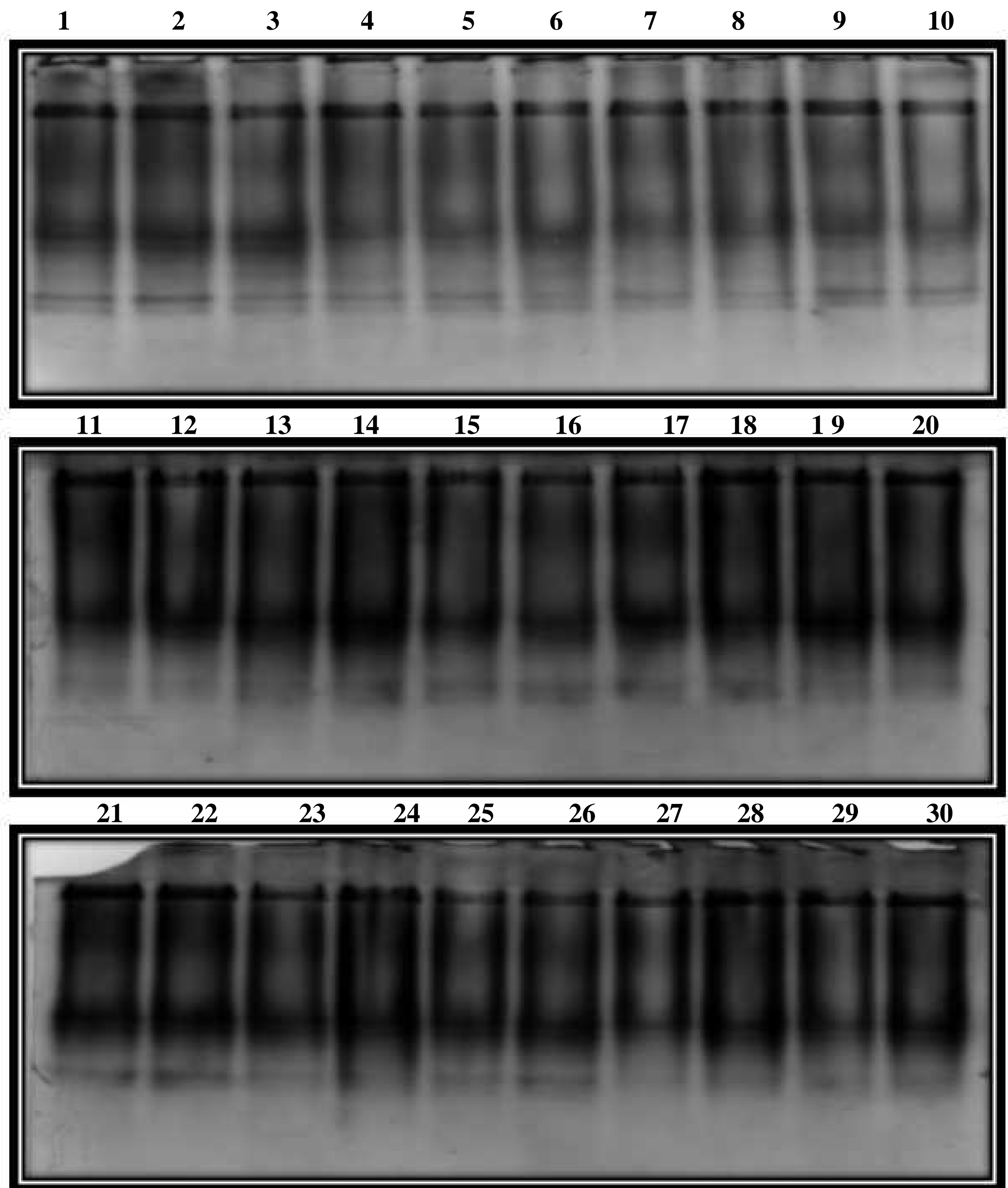


Plate 4.7 : Esterase from okra seedling

(**1**:AOL 12-52, **2**:AOL 14-32, **3**:AOL 13-94, **4**:AOL 09-02, **5**:AOL 14-29, **6**:JOL 11-12, **7**:AOL 13-141, **8**:JDNO 11-11, **9**:Kashi Kranti, **10**:JDNO 11-12, **11**: AOL 13-133, **12**:JOL 09-05, **13**:AOL 14-11, **14**:JOL 13-05, **15**:GP OK-296, **16**:GP OK-213, **17**:GP OK-292, **18**: GAO-5, **19**:Red Okra, **20**:Pusa Sawani, **21**:Parbhani Kranti, **22**:AOL 13-73, **23**:AOL 12-55, **24**:AOL 13-88, **25**: AOL 13-112, **26**:AOL 14-08, **27**:AOL 13-90 , **28**:AOL 12-59, **29**:JOL 13-07 and **30**:AOL 13-144)

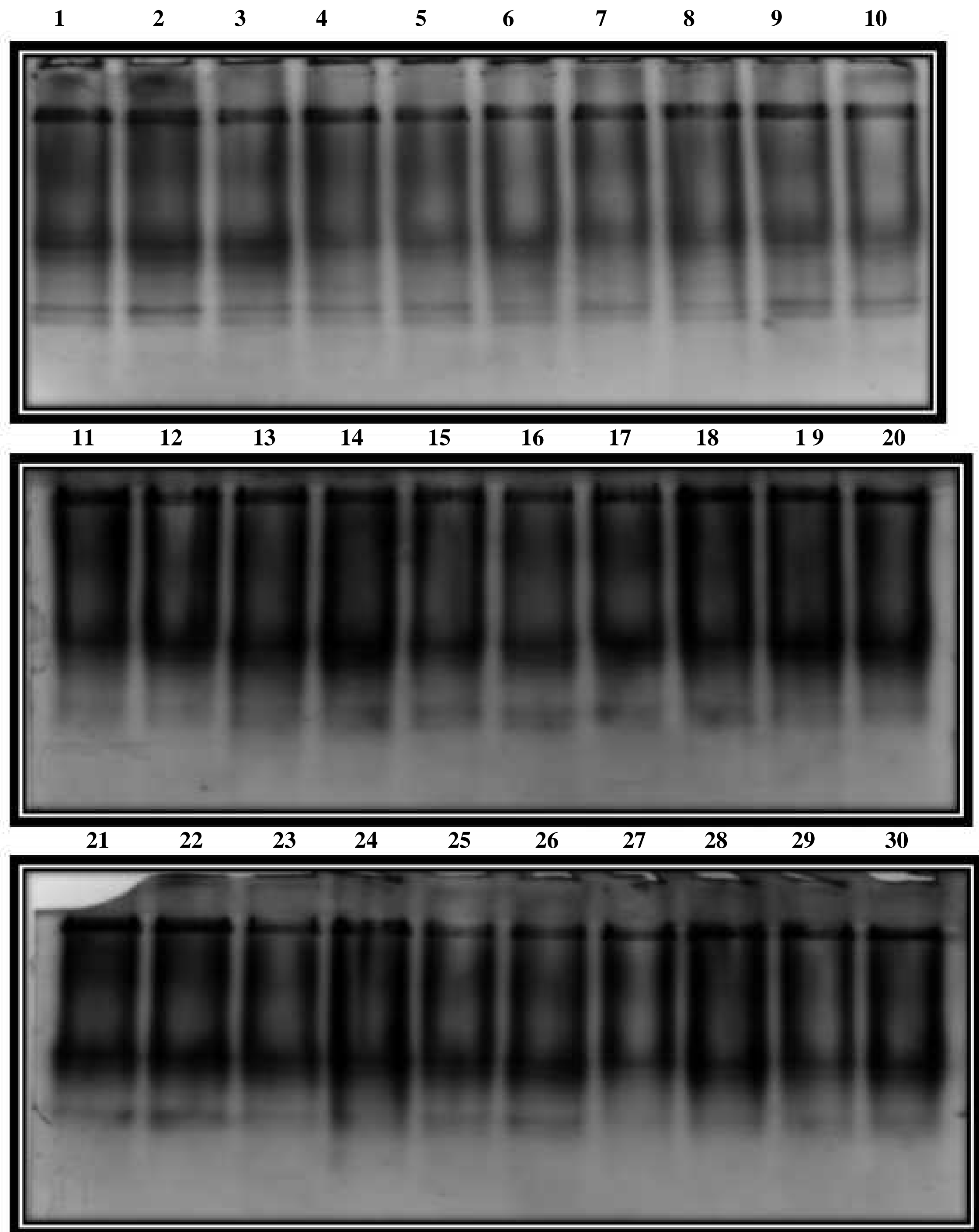


Plate 4.7: Esterase from okra seedling

(**1**:AOL 12-52, **2**:AOL 14-32, **3**:AOL 13-94, **4**:AOL 09-02, **5**:AOL 14-29, **6**:JOL 11-12, **7**:AOL 13-141, **8**:JDNO 11-11, **9**:Kashi Kranti, **10**:JDNO 11-12, **11**: AOL 13-133, **12**:JOL 09-05, **13**:AOL 14-11, **14**:JOL 13-05, **15**:GP OK-296, **16**:GP OK-213, **17**:GP OK-292, **18**: GAO-5, **19**:Red Okra, **20**:Pusa Sawani, **21**:Parbhani Kranti, **22**:AOL 13-73, **23**:AOL 12-55, **24**:AOL 13-88, **25**: AOL 13-112, **26**:AOL 14-08, **27**:AOL 13-90 , **28**:AOL 12-59, **29**:JOL 13-07 and **30**:AOL 13-144)

4.4.2 SDS-PAGE of okra seed protein

The total proteins were fractioned into fifteen bands among different genotypes (Fig.4.7). The maximum numbers of bands fifteen (15) were observed in GAO-5 followed by AOL 12-52 (12) and JOL 11-12 (12). Red okra was observed with 8 bands, it possessed minimum numbers of bands which differentiates from all thirty genotypes.

Torkpo *et al.,* (2006) analyzed 20 okra (*Abelmoschus esculentus* L.) accession diversity through total as well as seed storage proteins. A total of 34 reproducible and easily scorable bands were observed with the number of bands per accession ranging from 1 – 21.

Jaccard's similarity coefficient on the basis of presence and absence of bands was calculated for all possible pairs of thirty genotypes of okra (Table 4.8). The highest similarity index value was recorded (1.00), between AOL 13-73 and AOL 13-88, while the least similarity index value was recorded (0.23), between Red okra & AOL 13-88. The average similarity coefficient among cultivars was 0.69 (Table 4.8). Based on this similarity index a dendogram was prepared and shown in Fig 4.7.

Table 4.7: Banding pattern of SDS-PAGE

Genotypes		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Molecular weight (Kd)	Rm values																														
388.94	0.055	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-
310.30	0.100	-	+	+	+	-	+	-	-	-	+	+	+	-	-	-	-	-	+	-	+	+	+	+	+	-	+	+	+	+	+
229.61	0.159	+	+	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+
197.51	0.188	+	+	-	+	-	+	-	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
112.29	0.299	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-
83.09	0.358	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	-
59.21	0.424	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	+	+	+	+	+	+	+	-	+	+	+	+	+
47.24	0.469	-	-	-	+	-	+	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+
34.95	0.528	+	+	+	-	+	+	+	+	+	-	-	-	-	-	-	+	+	-	+	+	+	+	+	+	-	-	-	-	-	-
23.54	0.605	+	-	-	+	-	-	-	-	-	+	-	-	-	-	+	+	+	-	+	+	-	-	-	-	-	+	+	+	+	+
16.16	0.679	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	-	-	+	+	+	+	+	+
11.09	0.753	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+
7.19	0.838	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-
6.93	0.845	-	+	+	+	+	+	+	+	+	+	+	+	+	+	-	-	-	+	+	+	+	-	-	-	-	-	-	-	+	-
6.30	0.838	+	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	-	-	-	-	-	-	+	-	-	-	-	-

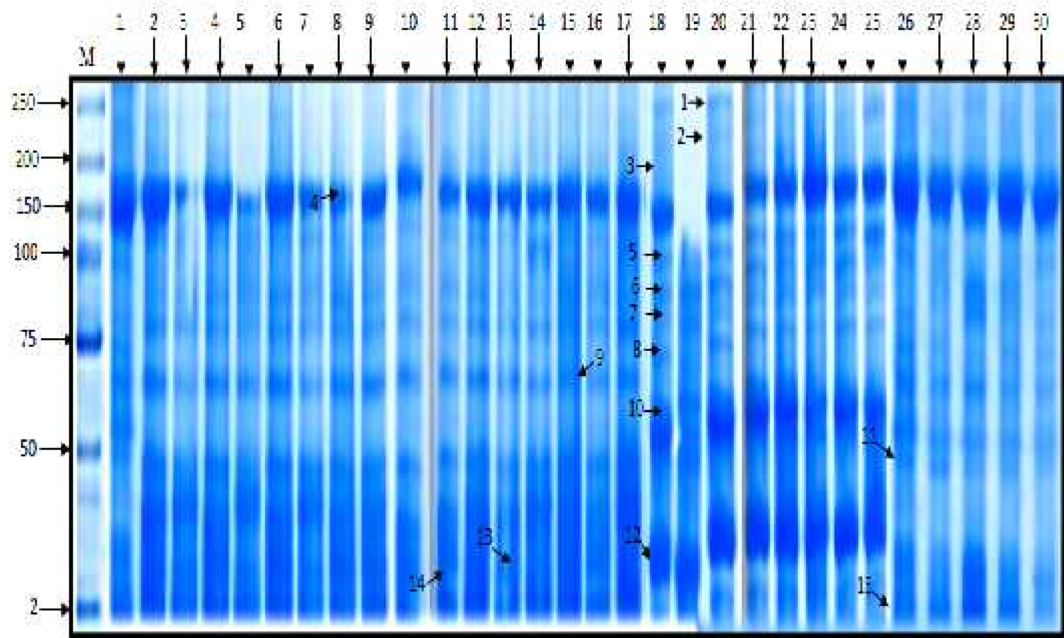


Plate 4.8: SDS PAGE of okra seed protein

M = Protein marker

1:AOL 12-52,	2:AOL 14-32,	3:AOL 13-94,	4:AOL 09-02,	5:AOL 14-29,	6:JOL 11-12
7:AOL 13-141,	8:JDNO 11-11,	9:Kashi Kranti,	10:JDNO 11-12,	11: AOL 13-123,	12:JOL 09-05
13:AOL 14-11,	14:JOL 13-05,	15:GP OK-296	16:GP OK-213,	17:GP OK-292,	18: GAO-5 ,
19:Red Okra,	20:Pusa Sawani,	21:Parbhani Kranti,	22:AOL 13-73,	23:AOL 12-55,	24:AOL 13-83,
25: AOL 13-112,	26:AOL 14-06,	27:AOL 13-90 ,	28:AOL 12-59,	29:JOL 13-07	30:AOL 13-144

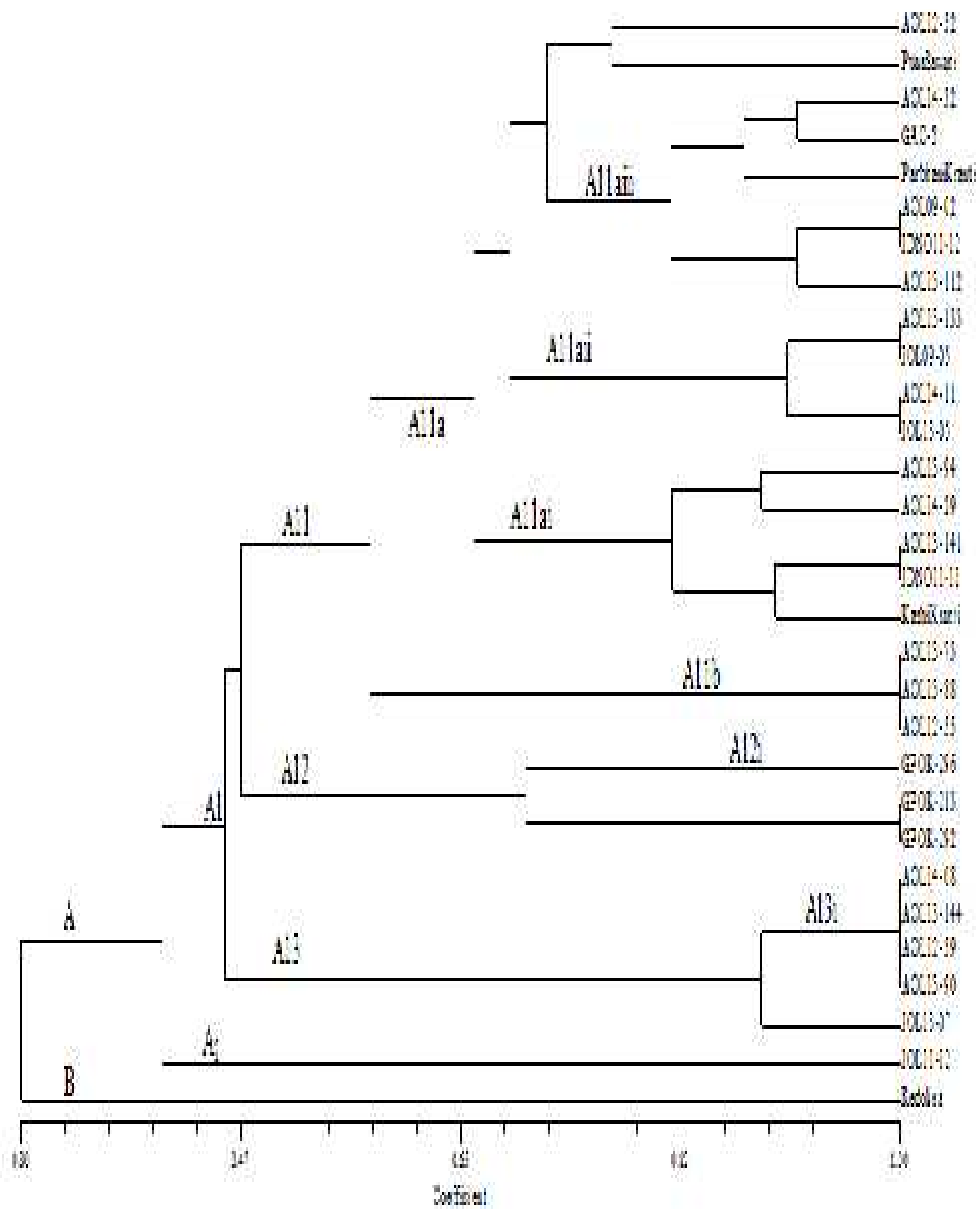


Fig. 4.7: Dendogram showing clustering of thirty okra genotypes constructed obtained from SDS-PAGE analysis

Table 4.8: Genetic similarity matrix of SDS-PAGE data based on Jaccard's similarity coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1.00																													
2	0.84	1.00																												
3	0.50	0.62	1.00																											
4	0.73	0.77	0.62	1.00																										
5	0.54	0.75	0.66	0.54	1.00																									
6	0.73	0.92	0.75	0.85	0.67	1.00																								
7	0.62	0.67	0.82	0.62	0.89	0.75	1.00																							
8	0.62	0.67	0.82	0.62	0.89	0.75	1.00	1.00																						
9	0.66	0.75	0.75	0.69	0.80	0.83	0.90	0.90	1.00																					
10	0.73	0.77	0.62	1.00	0.54	0.85	0.82	0.62	0.46	1.00																				
11	0.64	0.66	0.67	0.77	0.58	0.77	0.87	0.67	0.75	0.77	1.00																			
12	0.64	0.66	0.67	0.77	0.58	0.77	0.87	0.67	0.75	0.77	1.00	1.00																		
13	0.66	0.62	0.58	0.69	0.64	0.69	0.73	0.73	0.81	0.66	0.91	0.91	1.00																	
14	0.66	0.62	0.58	0.69	0.64	0.69	0.73	0.73	0.81	0.66	0.91	0.91	1.00	1.00																
15	0.62	0.45	0.58	0.62	0.42	0.50	0.50	0.50	0.58	0.62	0.67	0.67	0.73	0.73	1.00															
16	0.54	0.46	0.42	0.49	0.45	0.43	0.42	0.42	0.50	0.45	0.46	0.46	0.50	0.50	0.70	1.00														
17	0.54	0.46	0.42	0.49	0.45	0.43	0.42	0.42	0.50	0.45	0.46	0.46	0.50	0.50	0.70	1.00	1.00													
18	0.64	0.82	0.67	0.92	0.58	0.92	0.87	0.67	0.75	0.90	0.82	0.82	0.75	0.75	0.54	0.36	0.36	1.00												
19	0.58	0.50	0.60	0.46	0.67	0.46	0.60	0.60	0.58	0.46	0.58	0.58	0.42	0.42	0.33	0.36	0.36	0.38	1.00											
20	0.77	0.66	0.54	0.77	0.46	0.77	0.54	0.54	0.61	0.77	0.57	0.57	0.50	0.50	0.43	0.36	0.36	0.69	0.50	1.00										
21	0.64	0.82	0.67	0.77	0.58	0.92	0.87	0.67	0.75	0.77	0.66	0.66	0.62	0.62	0.43	0.36	0.36	0.83	0.36	0.85	1.00									
22	0.50	0.67	0.50	0.62	0.42	0.75	0.50	0.50	0.58	0.62	0.54	0.54	0.46	0.46	0.50	0.42	0.42	0.67	0.23	0.67	0.82	1.00								
23	0.50	0.67	0.50	0.62	0.42	0.75	0.50	0.50	0.58	0.62	0.54	0.54	0.46	0.46	0.50	0.42	0.42	0.67	0.23	0.67	0.82	1.00	1.00							
24	0.50	0.67	0.50	0.62	0.42	0.75	0.50	0.50	0.58	0.62	0.54	0.54	0.46	0.46	0.50	0.42	0.42	0.67	0.23	0.67	0.82	1.00	1.00	1.00						
25	0.42	0.25	0.08	0.30	0.18	0.31	0.27	0.17	0.22	0.30	0.35	0.35	0.36	0.36	0.40	0.30	0.30	0.33	0.09	0.25	0.29	0.27	0.27	0.27	1.00					
26	0.45	0.46	0.38	0.67	0.23	0.54	0.31	0.31	0.36	0.67	0.46	0.46	0.38	0.38	0.55	0.33	0.33	0.58	0.25	0.46	0.46	0.55	0.55	0.55	0.44	1.00				
27	0.45	0.46	0.38	0.67	0.23	0.54	0.31	0.31	0.36	0.67	0.46	0.46	0.38	0.38	0.55	0.33	0.33	0.58	0.25	0.46	0.46	0.55	0.55	0.55	0.44	1.00	1.00			
28	0.45	0.46	0.38	0.67	0.23	0.54	0.31	0.31	0.36	0.67	0.46	0.46	0.38	0.38	0.55	0.33	0.33	0.58	0.25	0.46	0.46	0.55	0.55	0.55	0.44	1.00	1.00	1.00		
29	0.50	0.54	0.58	0.75	0.31	0.62	0.38	0.38	0.44	0.75	0.54	0.54	0.46	0.46	0.50	0.31	0.31	0.67	0.38	0.54	0.54	0.50	0.50	0.50	0.40	0.86	0.89	0.86	1.00	
30	0.45	0.46	0.38	0.67	0.23	0.54	0.31	0.31	0.36	0.67	0.46	0.46	0.38	0.38	0.55	0.33	0.33	0.58	0.25	0.46	0.46	0.55	0.55	0.55	0.44	1.00	1.00	1.00	0.86	1.00

(1: AOL 12-52, 2: AOL 14-32, 3: AOL 13-94, 4: AOL 09-02, 5: AOL 14-25, 6: JOL 11-12, 7: AOL 13-141, 8: JDNO 11-11, 9: Kasi Kranti, 10: JDNO 11-12, 11: AOL 13-133, 12: JOL 09-05, 13: AOL 14-11, 14: JOL 13-05, 15: GP OK-296, 16: GP OK-213, 17: GP OK-292, 18: GAO-5, 19: Red China, 20: Pusa Sawani, 21: Faribadi Kranti, 22: AOL 13-73, 23: AOL 15, 24: AOL 13-88, 25: AOL 13-112, 26: AOL 14-08, 27: AOL 13-90, 28: AOL 12-59, 29: JOL 13-07 And 30: AOL 13-144)

4.4.2.1Clustering pattern of Total protein

The dendrogram generated on the principle of unweighted pairwise method using arithmetic average (UPGMA) was constructed and the genotypes were grouped in to one minor cluster and major cluster formed at a similarity coefficient of 0.30 (Fig.4.7).Minor cluster (B) includes only Red Okra genotype. Major cluster (A) was divided into two sub clusters A1 and A2. Sub-cluster A1 further separate into three clusters that is A11 ,A12 and A13.The A11 sub-cluster comprised of AOL12-52, Pusa Sawani, GAO-5, Parbhani Kranti, AOL 09-02, JDNO 11-11, AOL 13-112, AOL 13-133, JOL09-05, AOL 14-11, JOL 13-05, AOL 13-94, AOL 14-29, AOL 13-141,JDNO 11-11,AOL13-73, AOL 12-55, AOL 13-88 and Kashi Kranti together. Sub-cluster A12 includes GP OK-296, GP OK-213 and GP OK-292 .while sub-cluster A13 includes AOL 14-08, AOL 13-144, AOL 12-59, AOL 13-90, JOL 13-07 and JOL 11-12.

4.4.2.2 Principle Component Analysis

The principle component analysis (PCA) results (Fig. 4.8 & 4.9) almost coincided with the results of cluster analysis. The clustering pattern of 2D and 3D of PCA analysis were in accordance with the dendogram clustering pattern which showed that genotypes of okra *viz.*, Red okra differentiated among all thirty genotypes.

Fig. 4.8: Two dimensional plot of SDS-PAGE

Fig.4.9: Three dimensional plot of SDS-PAGE

4.5: Qualitative and Quantitative analysis of genomic DNA

In present investigation, the average concentration of DNA extracted from okra leaves was quantified on nanodrop spectrophotometer (Table 4.9). The genotype GP OK-292 and AOL14-32 showed highest concentration of DNA 1328.6 and 1321.8 ng/µl, respectively. Genotype JDNO 11-12 showed the lowest concentration of DNA 200.3 ng/µl.

The quality of DNA was determined at 260/280 and 260/230 ratio. DNA thus extracted was utilized for molecular markers study through RAPD and SSR. The results presented in this chapter are according to the amplification patterns of molecular markers described below:

Table 4.9 Qualitative and Quantitative analysis of genomic DNA:

Sr. No.	Genotype	Stock solution (ng/µl)	Absorbance 260/280	Preparation of working solution(20 ng/µl, 100µl)	
				Stock solution taken (µl)	Water (Nuclease free) added (µl)
1	AOL-12-52	795.3	1.90	13.27	186.72
2	AOL-14-32	1321.8	1.90	7.56	192.43
3	AOL-13-94	436.9	1.90	22.88	177.11
4	AOL-09-02	563.5	1.80	17.74	182.25
5	AOL-14-29	740.8	1.88	13.49	186.50
6	JOL-11-12	280.9	1.92	35.59	164.40
7	AOL-13-141	329.2	1.85	30.37	169.63
8	JDNO-11-11	491.8	1.89	20.33	179.66

Sr. No.	Genotype	Stock solution (ng/µl)	Absorbance 260/280	Preparation of working solution(20 ng/µl, 100µl)	
9	KASHI KRANTI	528.7	1.82	18.91	181.08
10	JDNO-11-12	200.3	1.85	50.17	149.82
11	AOL-13-133	517.3	1.80	19.32	180.67
12	JOL-09-05	305.9	1.93	32.69	167.30
13	AOL-14-11	1075.4	1.87	9.30	190.69
14	JOL-13-05	555.7	1.84	`18.01	181.98
15	GP-OK-296	833.9	1.86	11.99	188.0
16	GP-OK-213	1030.2	1.80	12.20	187.80
17	GP-OK-292	1328.6	1.89	7.52	192.47
18	GAO-5	512.6	1.85	18.27	181.73
19	Red okra	726.4	1.94	13.76	186.23
20	Pusa Sawani	769.1	1.94	13.00	187.00
21	Parbhani Kranti	758.4	1.91	13.18	186.81
22	AOL-13-73	1023.1	1.95	9.77	190.23
23	AOL-12-55	523.3	1.94	19.10	180.90
24	AOL-13-88	569.9	1.90	17.86	182.14
25	AOL-13-112	1308.6	1.82	7.64	192.35
26	AOL-14-08	935.1	1.85	10.69	189.30
27	AOL-13-90	891.4	1.90	11.21	188.78
28	AOL-12-59	927.9	1.87	10.77	189.22
29	JOL-13-07	417.1	1.90	23.97	176.02
30	AOL-13-144	841.3	1.89	11.88	188.11

4.5.1 RAPD Marker analysis

The existence of genetic variation among the genotypes which can be easily identified through molecular markers like RAPD, which detects variation at the DNA level. The RAPD analysis was carried out in order to analyze molecular characterization of all okra genotypes. As mentioned in section 3.10.4, various bio-statistical parameters were calculated which are efficient for diversity analysis. They include PIC value, PCA (principle component analysis), Marker Index, assay efficiency index and similarity index.

Table 4.10: Polymorphism pattern of amplified RAPD primers by thirty okra genotypes

No.	Locus Name	Molecular size range (bp)	Total no. of bands	No. of Loci	No. of Polymorphic loci	No. of Monomorphic loci	Polymor-phism (%)	PIC Value
1	OPC-8	185-1758bp	220	11	8	3	72.7	0.880
2	OPC-19	224-1852bp	132	6	3	3	50.0	0.794
3	OPX-17	152-1643bp	137	9	7	2	77.8	0.810
4	OPY-02	281-1239bp	172	10	7	3	70.0	0.858
5	UBC -509	405-1344bp	107	7	4	3	57.1	0.756
6	OPY-04	230-1132bp	111	12	11	1	91.7	0.844
7	OPAE-15	341-1703bp	108	9	7	2	77.8	0.800
8	UBC-465	371-1926bp	87	8	8	0	100.0	0.826
Total		**-**	**1074**	**72**	**55**	**17**	**-**	**-**
Average		**273-1574bp**	**134.2**	**9**	**6.87**	**2.12**	**74.6**	**0.821**

4.5.1.2 Polymorphism Pattern of RAPD

The data from random amplification of polymorphic DNA with 8 primers produced total bands were 1074. Out of the 72 loci produced, 55 were polymorphic and 17 were monomorphic, hence the total polymorphism percentage was found to be 74.60% (Table 4.13).where as Samarajeewa and Rathnayaka, (2004) obtained 73.84% polymorphism in okra. The average

Polymorphism Information content (PIC) value for RAPD was 0.821. Average number of loci per primer was found to be 9.0 average number of polymorphic loci obtained per primer (Assay Efficiency index) was found to be 6.87. The molecular size of the amplified PCR products ranged from 152bp (OPX-17) to 1926bp (UBC-465) (Table 4.10). Results are in line with previous studies by Prakash *et al.*, (2011).

The highest similarity index value 0.91 was found between AOL 13-90 and AOL 12-59, while the least similarity index value 0.45 was found between JOL09-05 and Red okra. The average similarity coefficient among genotypes was 0.59 (Table 4.11).

The minimum (87) and maximum (220) number of bands were produced by UBC-465 and OPC-8, respectively. The highest polymorphism (100%) was produced by UBC-465 (Table 4.10). The highest PIC value obtained was 0.880 for OPC-8 and lowest PIC value obtained was 0.756 for UBC-509. Marker Index values were calculated according to the formulas described in section 3.10.4 Marker Index value for polled RAPD data was found to be 4.07. The high value of Marker Index can be justified due to high value of Effective Multiplex Ratio (EMR) component. EMR value is affected by the number of polymorphic and total number of loci amplified. Based on the RAPD data, cluster analysis was performed using genetic similarity values and a dendrogram was generated showing the grouping of genotypes according genetic diversity.

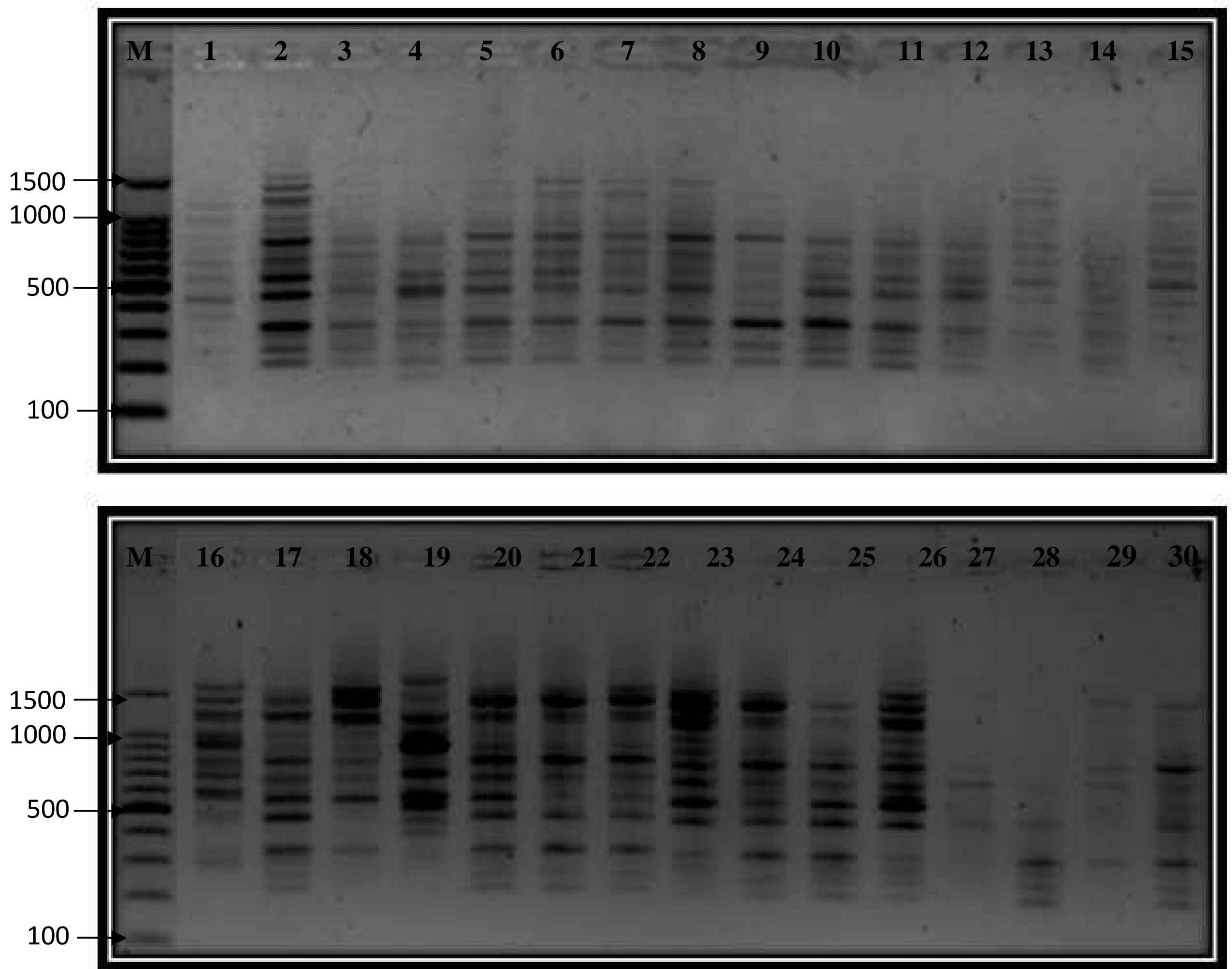


Plate 4.9: RAPD profile of OPC-8

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

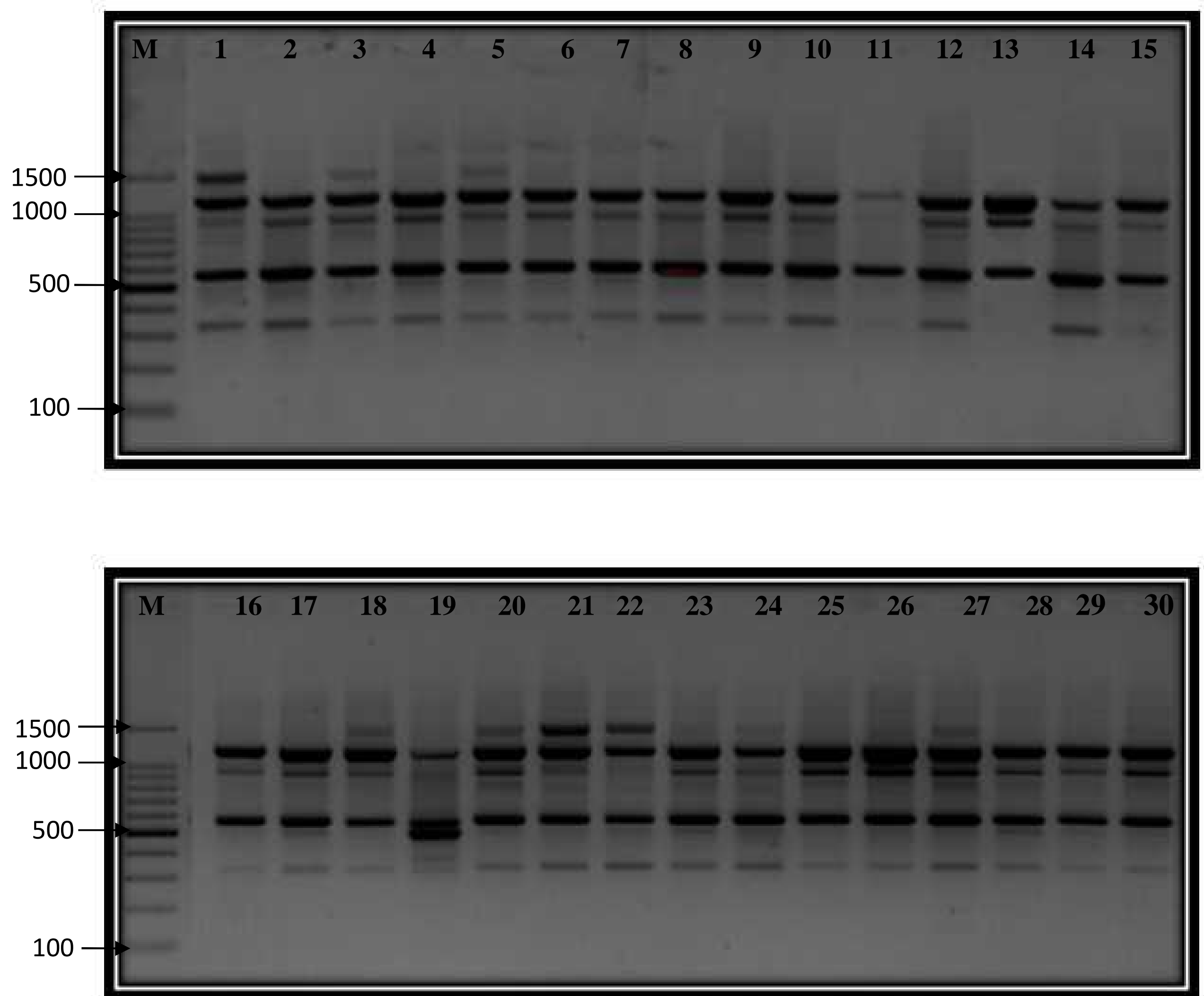


Plate 4.10: RAPD profile of OPC-19

M = DNA marker

((1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

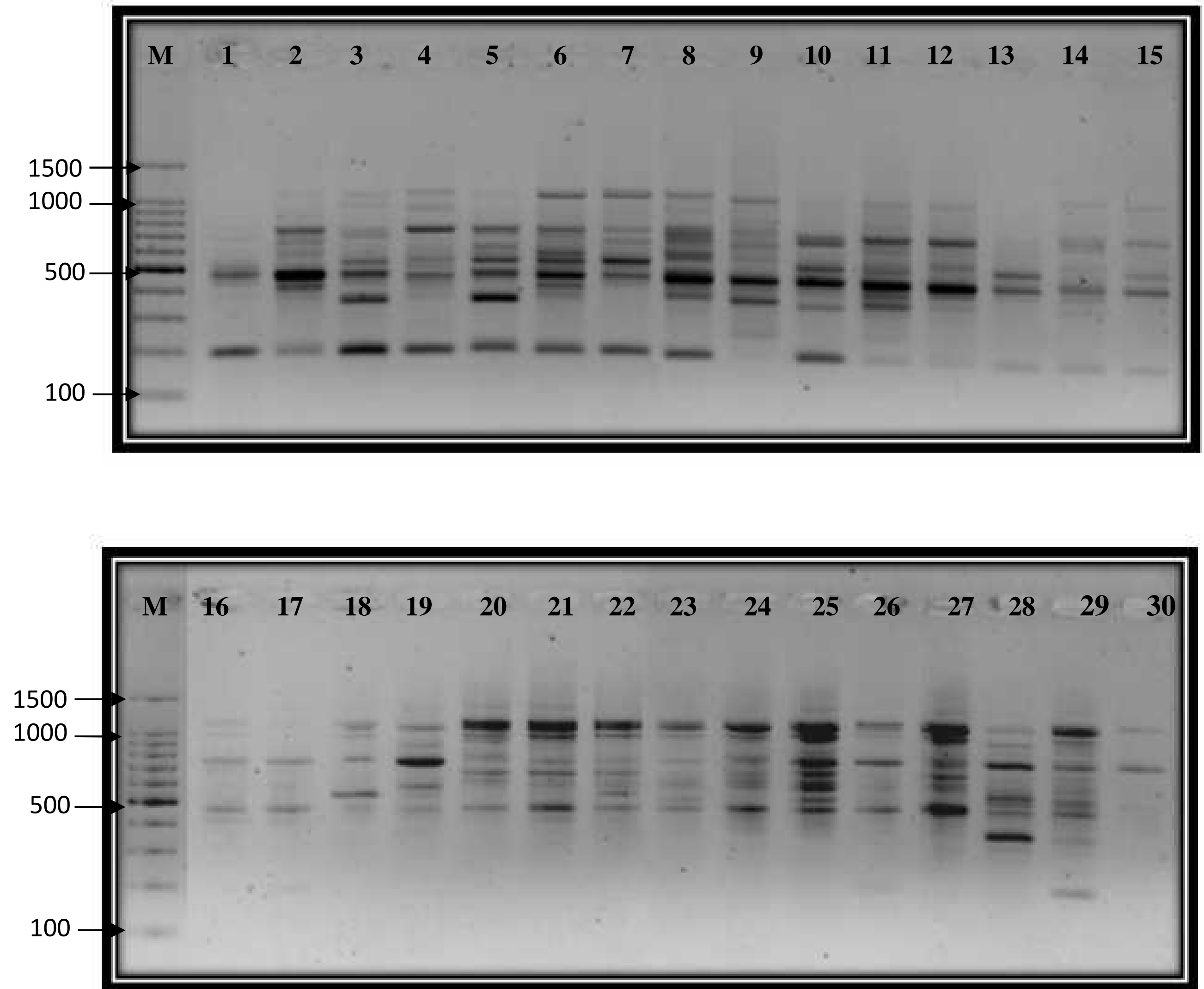


Plate 4.11: RAPD profile of OPX-17

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

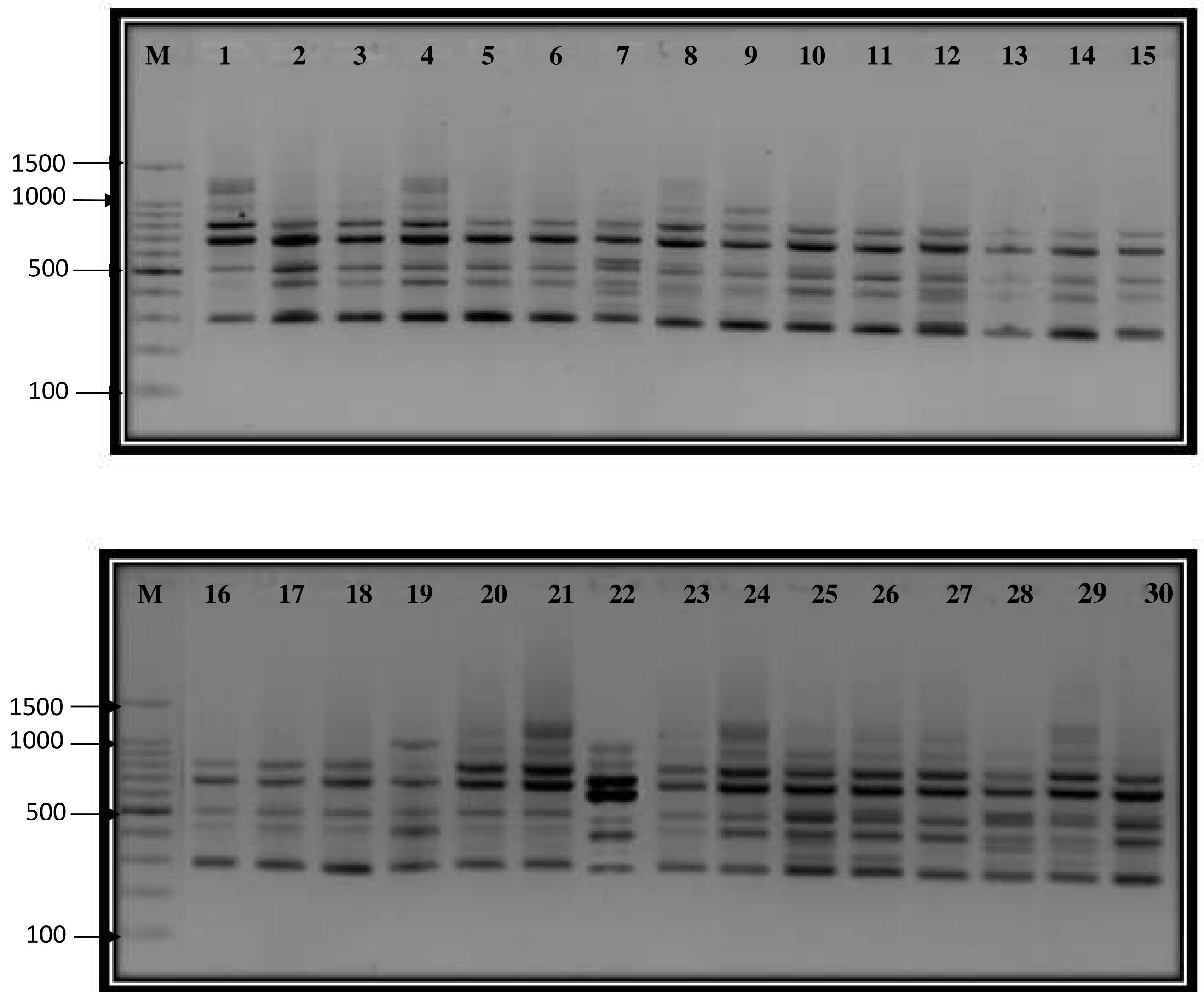


Plate 4.12: RAPD profile of OPY-2

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

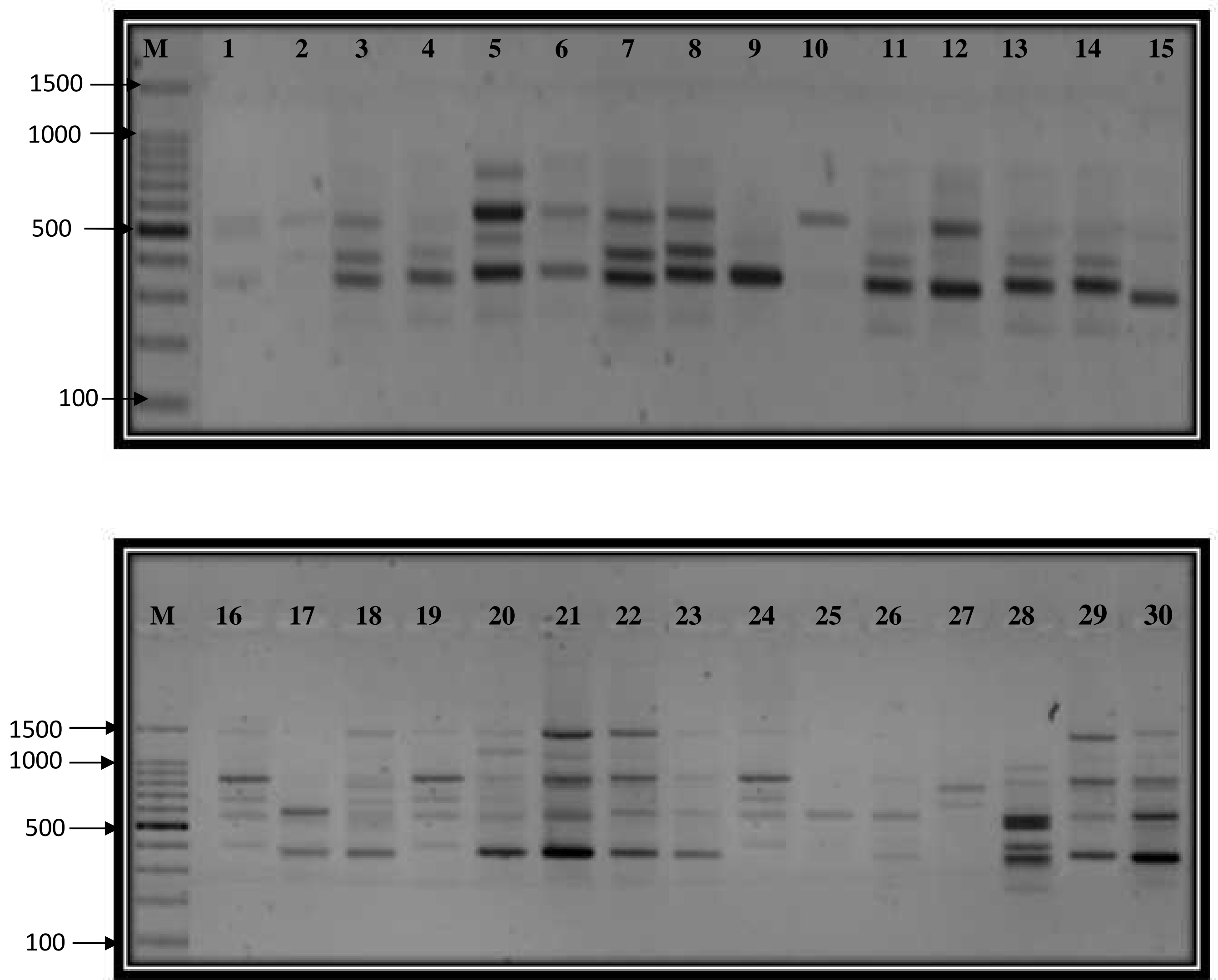


Plate 4.13: RAPD profile of UBC-509

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

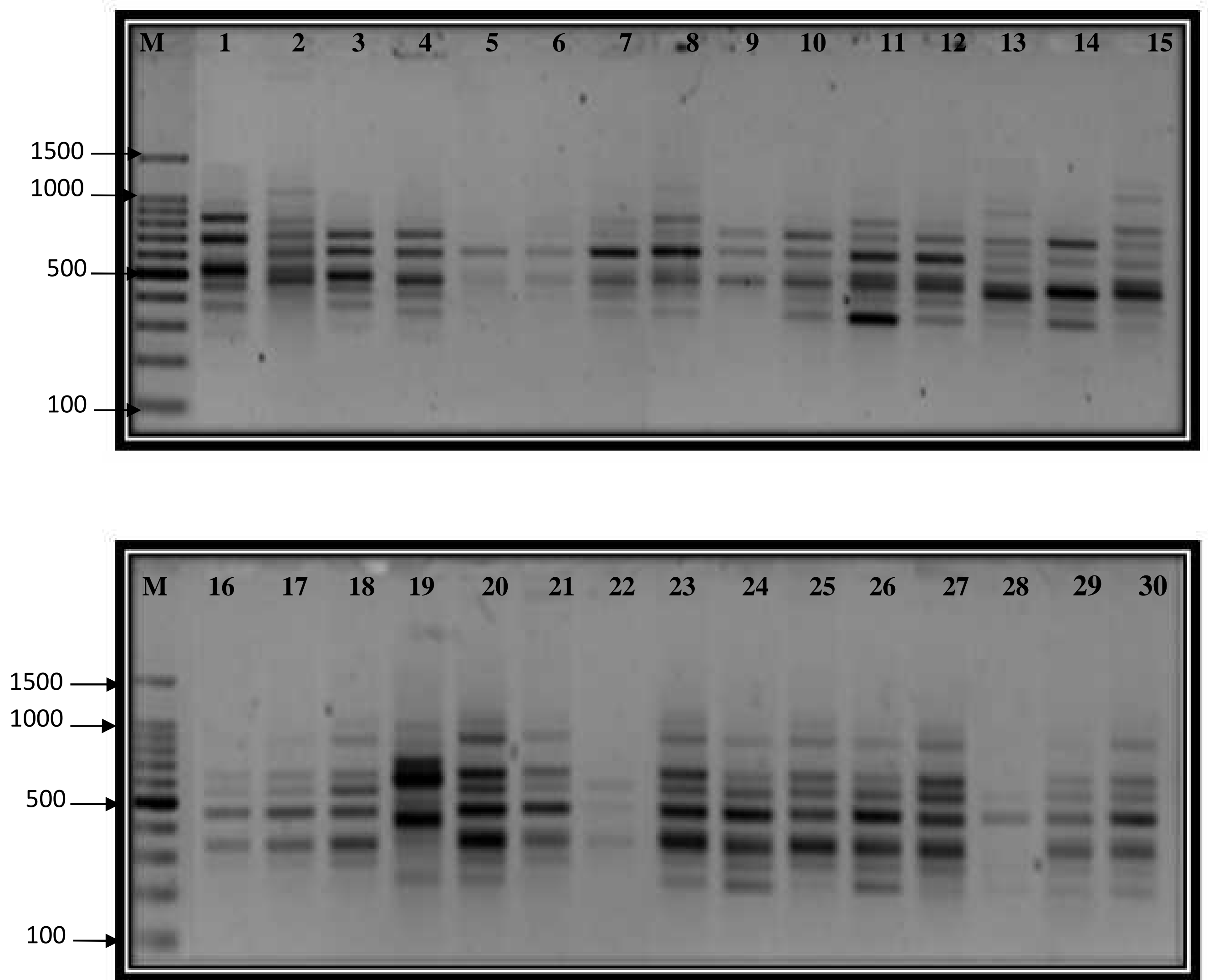


Plate 4.14: RAPD profile of OPY-4

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

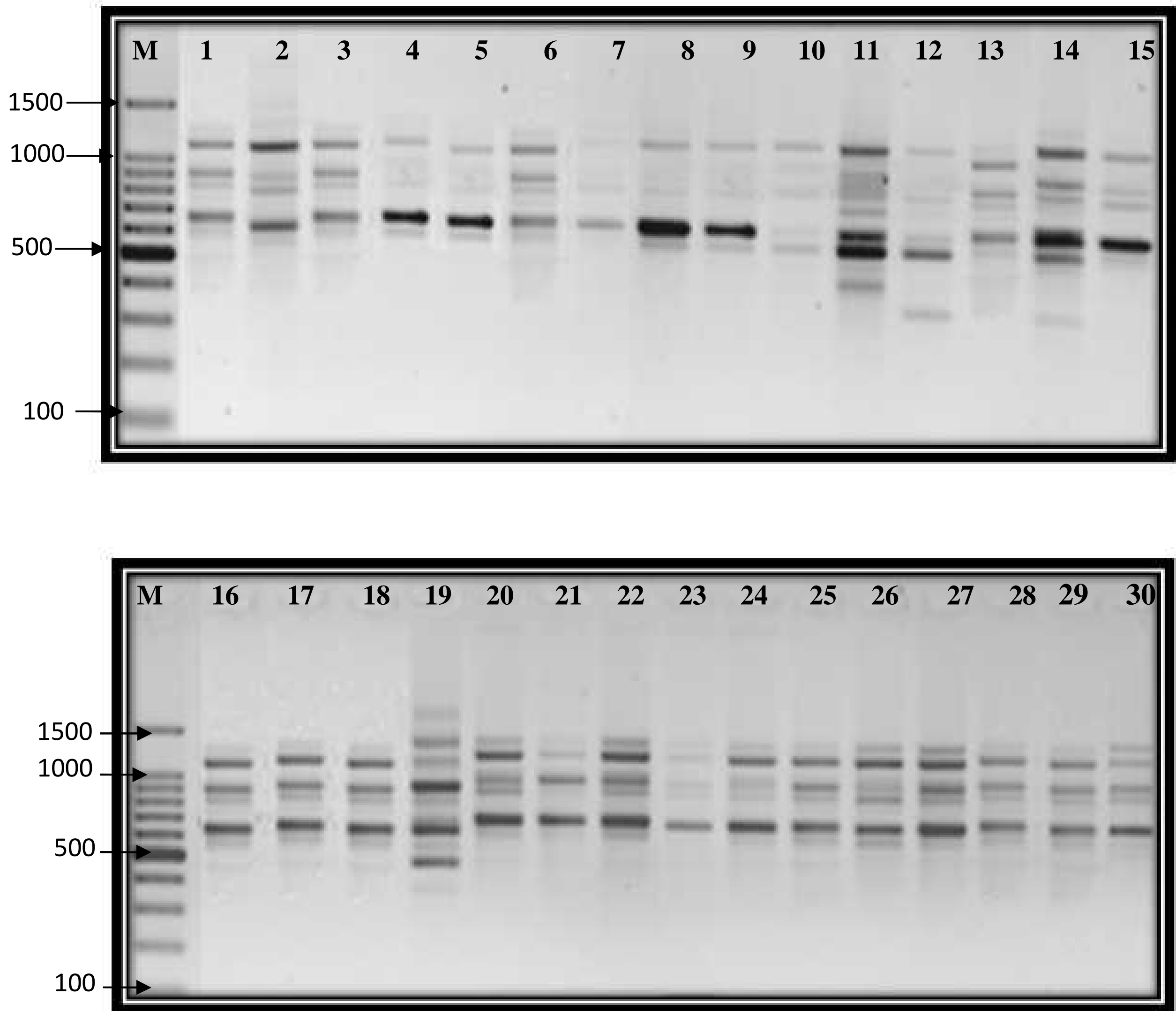


Plate 4.15: RAPD profile of OPAE-15

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

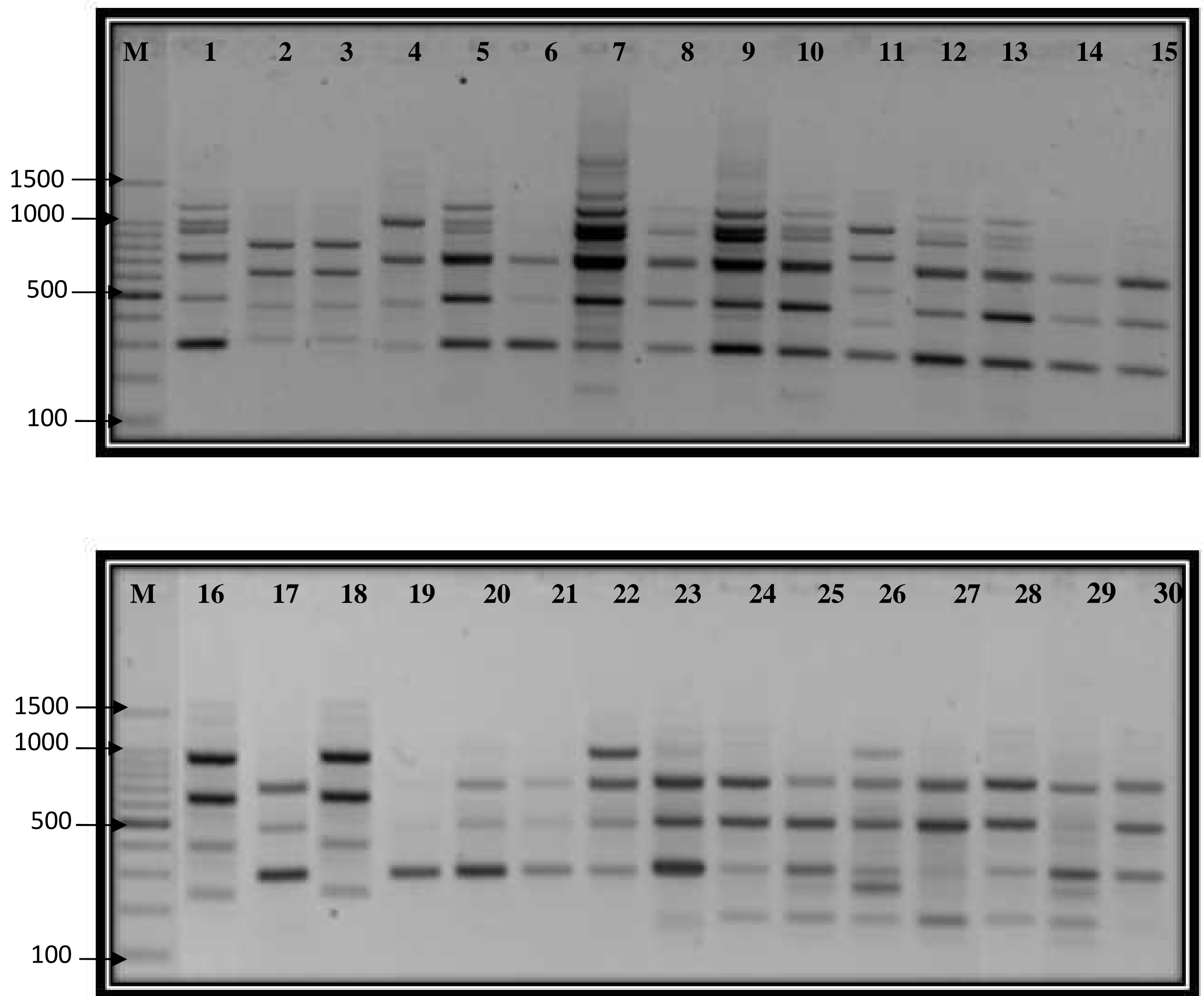


Plate 4.16: RAPD profile of UBC-465

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

4.5.1.3 Clustering Pattern for RAPD

The Clustering pattern of thirty okra genotypes was generated based on UPGMA-dendogram using the pooled molecular data of eight RAPD loci and presented in Fig 4.10. The results indicated that two clusters namely A and B were formed at a similarity coefficient of 0.59. Cluster A was divided into two sub-cluster A_1 and A_2. A_1 cluster further separate into A_{1a} and A_{1b} which is consisted of AOL 13-144,AOL 14-32,AOL13-94, AOL09-02, JOL11-12, JDNO 11-11, AOL14-29, AOL 13-141, Kashi kranti, GAO-5,Pusa Sawani, AOL 12-52,AOL 13-73, AOL 12-55, AOL 13-88, AOL 13-112, AOL 13-90, AOL 12-59, AOL 14-08, JOL 13-07, JOL 09-05, while A2 sub-cluster consisted of JDNO 11-12,AOL 13-133,Parbhani Kranti, AOL 14-11, JOL 13-05, GP OK-296, GP OK-213 ,GP OK -292.Major cluster B consisted of Red okra.

4.5.1.4 Principle Component Analysis of RAPD

The principle component analysis (PCA) results (Fig. 4.11 and 4.12) almost coincided with the results of cluster analysis. The clustering pattern of 2D and 3D of PCA analysis were in accordance with the dendogram clustering pattern which showed that genotype Red okra were differentiated among all thirty genotypes, It shows that 2D and 3D plots gives same results like dendrogram (Fig.4.10). The results of RAPD analysis of thirty okra genotypes with eight random primers revealed that this dominant marker can efficiently differentiate genotypes on the basis of genetic variation.

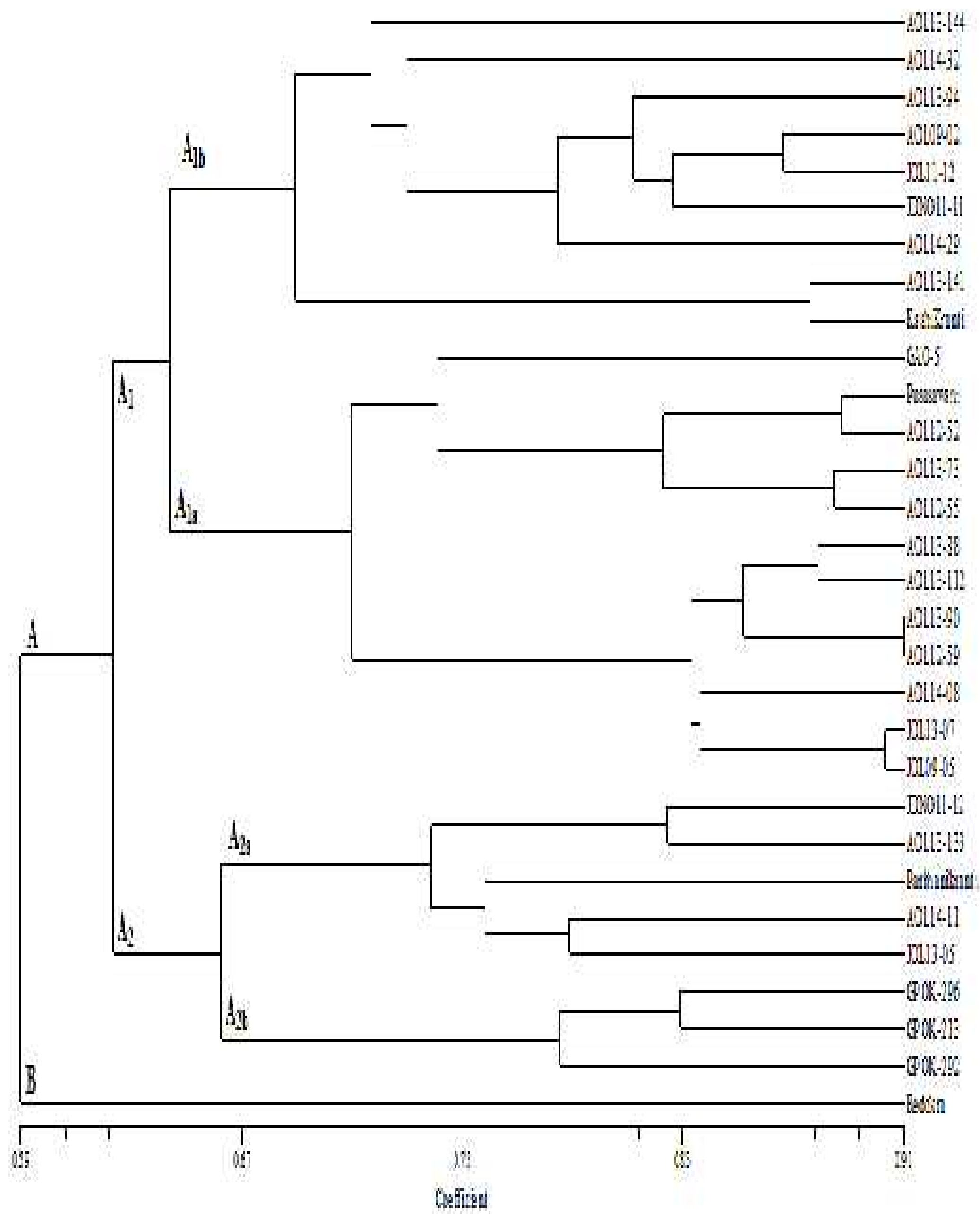


Fig. 4.10: Dendogram showing clustering of thirty okra genotypes constructed using UPGMA based on Jaccard's coefficient obtained from RAPD analysis

Table 4.11: Genetic similarity matrix of RAPD data based on Jaccard's similarity coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1.00																													
2	0.67	1.00																												
3	0.71	0.77	1.00																											
4	0.75	0.69	0.84	1.00																										
5	0.78	0.67	0.81	0.82	1.00																									
6	0.70	0.75	0.81	0.85	0.79	1.00																								
7	0.64	0.64	0.69	0.70	0.71	0.75	1.00																							
8	0.67	0.77	0.78	0.79	0.71	0.88	0.82	1.00																						
9	0.62	0.59	0.67	0.67	0.69	0.66	0.83	0.75	1.00																					
10	0.69	0.70	0.67	0.63	0.74	0.65	0.71	0.71	0.73	1.00																				
11	0.64	0.65	0.62	0.59	0.63	0.60	0.62	0.62	0.63	0.82	1.00																			
12	0.58	0.65	0.67	0.63	0.65	0.61	0.59	0.67	0.60	0.73	0.77	1.00																		
13	0.58	0.71	0.68	0.60	0.62	0.66	0.63	0.68	0.53	0.65	0.74	0.74	1.00																	
14	0.60	0.74	0.66	0.63	0.64	0.68	0.62	0.70	0.56	0.72	0.82	0.77	0.79	1.00																
15	0.64	0.73	0.57	0.55	0.60	0.63	0.54	0.65	0.49	0.63	0.66	0.67	0.63	0.80	1.00															
16	0.59	0.63	0.60	0.57	0.59	0.68	0.53	0.60	0.43	0.62	0.56	0.66	0.71	0.69	0.83	1.00														
17	0.59	0.75	0.65	0.60	0.53	0.70	0.60	0.68	0.54	0.61	0.60	0.65	0.70	0.68	0.76	0.82	1.00													
18	0.61	0.59	0.55	0.53	0.57	0.57	0.50	0.56	0.43	0.53	0.49	0.53	0.57	0.56	0.61	0.65	0.61	1.00												
19	0.60	0.60	0.52	0.56	0.48	0.57	0.47	0.55	0.46	0.47	0.46	0.45	0.47	0.52	0.55	0.48	0.60	0.66	1.00											
20	0.73	0.70	0.64	0.65	0.59	0.66	0.55	0.64	0.53	0.55	0.54	0.52	0.55	0.61	0.64	0.56	0.70	0.68	0.32	1.00										
21	0.73	0.70	0.67	0.63	0.61	0.70	0.57	0.67	0.52	0.54	0.53	0.54	0.55	0.60	0.64	0.59	0.70	0.66	0.73	0.89	1.00									
22	0.70	0.70	0.67	0.64	0.70	0.74	0.67	0.76	0.65	0.61	0.57	0.54	0.55	0.60	0.64	0.59	0.70	0.68	0.66	0.80	0.81	1.00								
23	0.72	0.70	0.65	0.67	0.61	0.68	0.63	0.71	0.61	0.61	0.57	0.54	0.55	0.60	0.63	0.59	0.69	0.67	0.73	0.85	0.84	0.88	1.00							
24	0.67	0.68	0.65	0.62	0.67	0.71	0.65	0.68	0.60	0.63	0.62	0.59	0.60	0.70	0.65	0.64	0.76	0.68	0.61	0.74	0.75	0.85	0.79	1.00						
25	0.70	0.70	0.60	0.61	0.61	0.70	0.67	0.71	0.53	0.65	0.64	0.58	0.62	0.73	0.67	0.59	0.70	0.58	0.63	0.75	0.76	0.77	0.75	0.88	1.00					
26	0.66	0.74	0.60	0.57	0.61	0.66	0.64	0.71	0.61	0.69	0.64	0.61	0.53	0.73	0.71	0.68	0.66	0.58	0.60	0.69	0.76	0.77	0.75	0.85	0.85	1.00				
27	0.67	0.65	0.58	0.56	0.60	0.64	0.65	0.66	0.57	0.63	0.59	0.60	0.57	0.66	0.65	0.57	0.64	0.60	0.59	0.71	0.67	0.74	0.70	0.84	0.85	0.82	1.00			
28	0.67	0.67	0.61	0.62	0.63	0.70	0.72	0.72	0.63	0.70	0.65	0.66	0.63	0.69	0.64	0.60	0.70	0.59	0.61	0.70	0.67	0.74	0.73	0.84	0.85	0.82	0.81	1.00		
29	0.72	0.65	0.62	0.67	0.57	0.68	0.63	0.74	0.57	0.64	0.59	0.60	0.57	0.71	0.70	0.61	0.63	0.60	0.63	0.79	0.76	0.72	0.73	0.81	0.84	0.84	0.84	0.84	1.00	
30	0.67	0.65	0.58	0.62	0.60	0.67	0.62	0.73	0.56	0.63	0.58	0.63	0.55	0.70	0.73	0.68	0.71	0.68	0.61	0.71	0.67	0.71	0.70	0.81	0.83	0.83	0.84	0.84	0.90	1.00

(1:AOL 12-52, 2:AOL 14-32, 3:AOL 13-94, **4** AOL 09-02, 5 AOL 14-29, 6 JOL 11-12, 7 AOL 13-141, 8:JDNO 11-11, 9:Kashi Kranti, **10** JDNO 11-12, 11 AOL13-133, **12** JOL 09-05, 13 AOL 14-11, 14:JOL 13-05, 15 GP OK-196, 16GP OK-213, 17GP OK-292, **18** GAO-5, 19:Red Okra, 20:Pusa Sawani, 21 Parbhani Kranti, 22:AOL 13-73, 23:A OL 12-56, 24:AOL 13-53, 25: AOL 13-112, 26 AOL 14-06, 27:AOL 13-90 , 28:AOL 12-59, 29 JOL 13-07 and 30:AOL 13-144)

Fig. 4.11: Two dimensional plot of RAPD

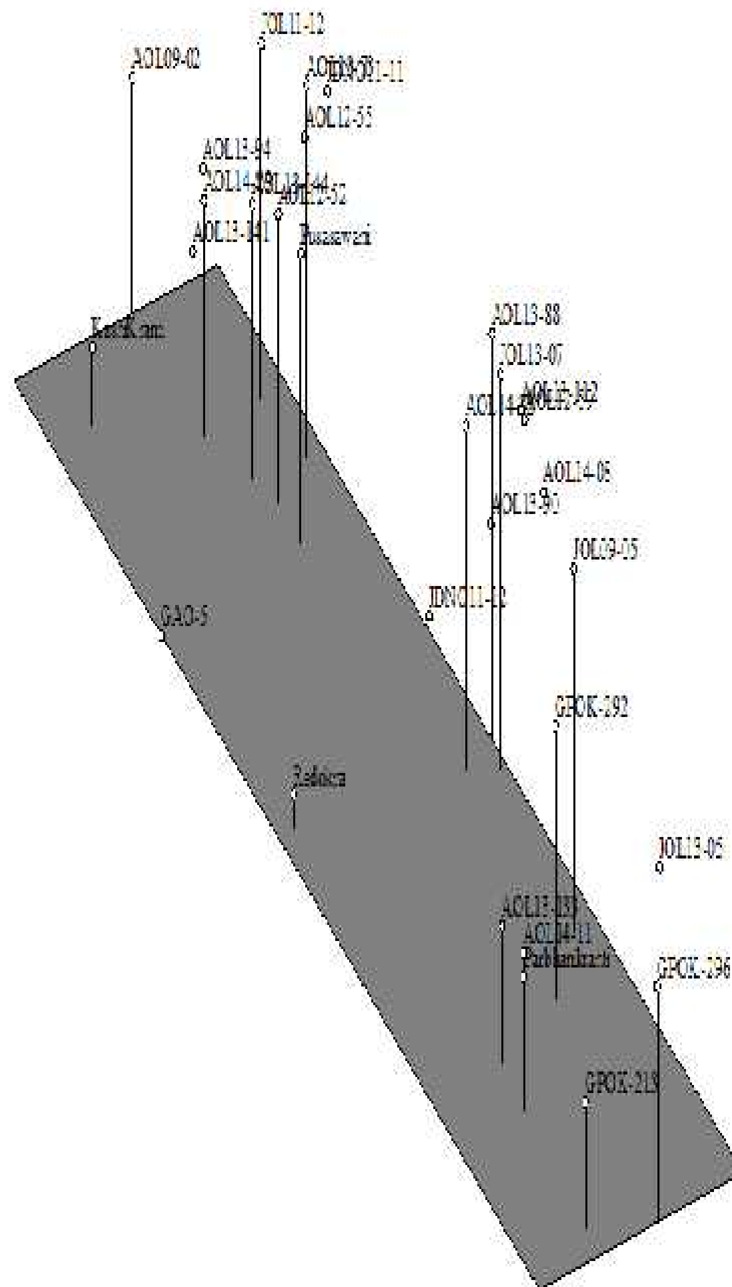


Fig. 4.12: Three dimensional plot of RAPD

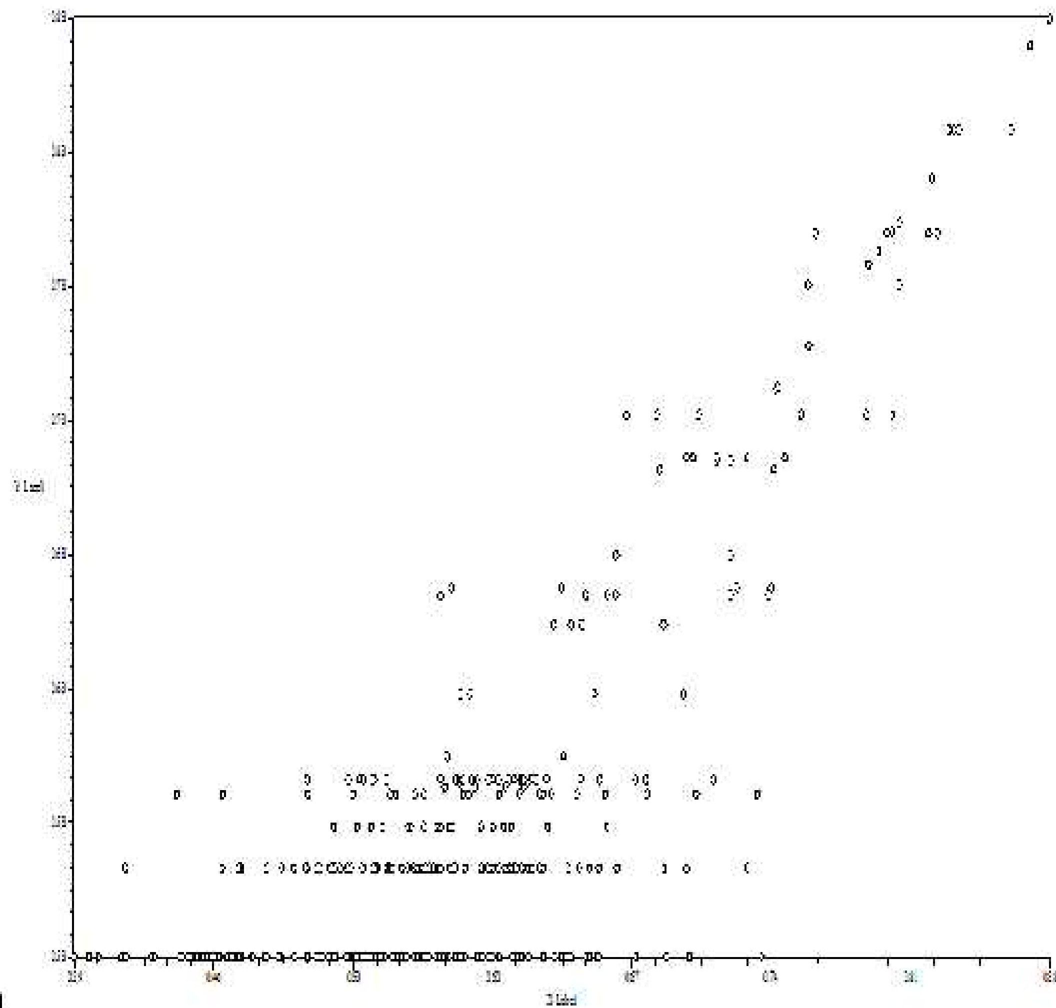


Fig. 4.13: Scatter -plot showing cophenetic correlation of RAPD

4.5.1.5 Cophenetic correlation study

Correlation study was carried out to compare the correlation of original similarity matrix of RAPD results with the dendrogram clustering pattern. Using the COPH module of NTSYSpc version 2.02, r value was calculated and results were also expressed graphically (Fig 4.13). High correlation between the similarity matrix and dendrogram pattern was justified by the 'r' value which was found to be 0.831 which is good to fit.

4.5.2 SSR markers analysis

In present investigation, thirty okra genotypes were subjected to SSR analysis using different primers of OK series. From those 15 primers *viz.,* OK-1, OK-3, OK-5, OK-6, OK-9, OK-10, OK-13, OK-16, OK-18, OK-21, OK-22, OK-23, OK-24, OK-27, OK-53 were amplified. As mentioned in section 3.10.2, various bio-statistical parameters were calculated, which are efficient for diversity analysis. The SSR primers have been designed from the EST sequences of transcriptome data carried out at the Anand Agricultural University, Anand.

4.5.2.1 Individual SSR primer profile analysis

4.5.2.1.1 OK-1

The primer OK-1 produced a total of three alleles ranging in size from 212 bp to 226 bp (Plate: 4.17). These three alleles were designated as 'A', 'B', and 'C'. Out of these three alleles, allele 'A' and allele 'C' recorded the highest & lowest allele frequency (Table 4.13). Allele 'A' (223 bp) was observed in genotypes *viz.,* GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa

Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55, AOL 13-88, AOL 13-112, AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 and AOL 13-144; allele 'B' (212 bp) was observed in genotypes *viz*., JDNO 11-11, Kashi Kranti, JDNO 11-12, AOL 13-133, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296 while allele 'C' (226 bp) was observed in genotypes *viz*., AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 and AOL 13-141. The PIC value for this marker was found to be 0.42. The expected heterozygosity in the present marker was 0.624 (Table 4.12).

4.5.2.1.2 OK-2

The primer OK-2 produced a total of three alleles ranging in size from 101 bp to 134 bp (Plate: 4.18). These three alleles were designated as 'A', 'B', 'C'. Among three alleles, allele 'A' recorded the highest allele frequency as compare to 'B' and 'C' (Table 4.11). Allele 'A' (119 bp) was observed in genotypes *viz.,* AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12, AOL 13-141, JDNO 11-11, Kashi Kranti, JDNO 11-12, AOL 13-133, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296; allele 'B' (134 bp) was observed in *viz.,* GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55 and AOL 13-88;while allele 'C'(101 bp) was observed in *viz.,* AOL 13-112, AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 and AOL 13-144. The PIC value for this marker was found to be 0.44. The expected heterozygosity in the present marker was 0.620 (Table 4.12).

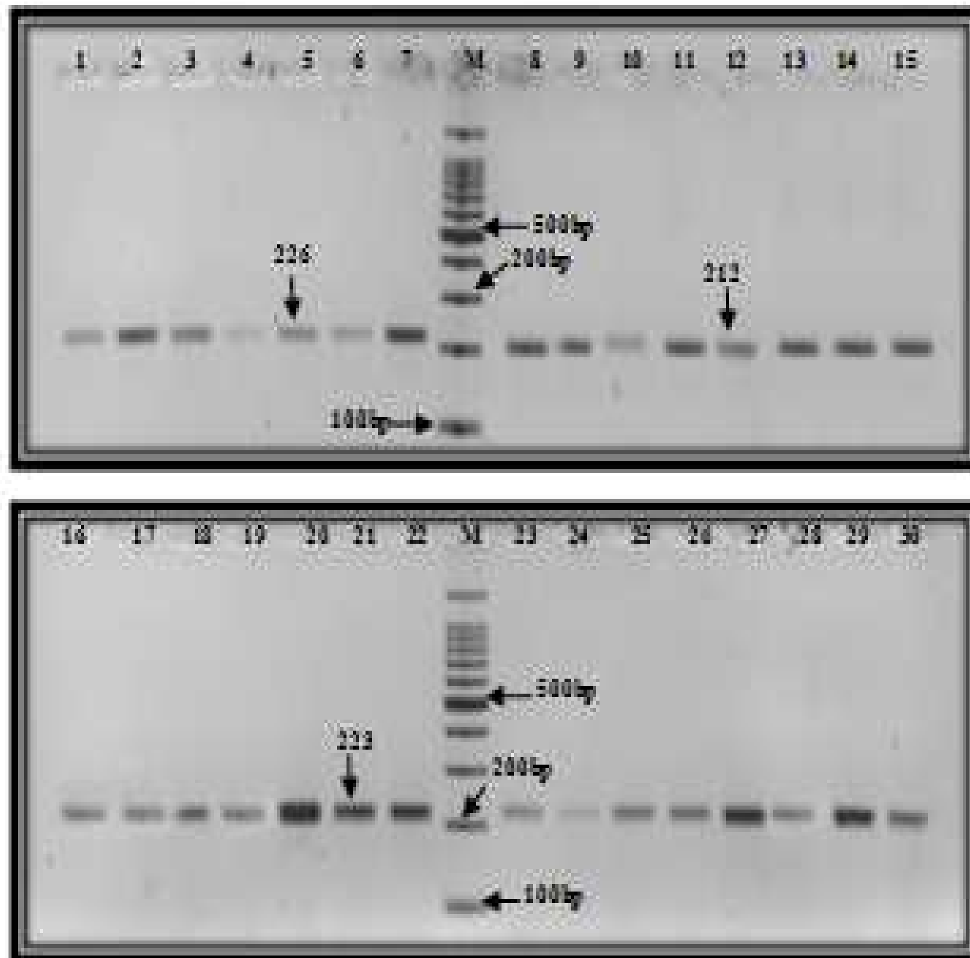


Plate 4.17: SSR profile of OK-1

M = DNA marker

(1) AOL 12-52	(2) AOL 14-32	(3) AOL 13-94	(4) AOL 09-02
(5) AOL 14-29	(6) JOL 11-12	(7) AOL 13-141	(8) JDNO 11-11
(9) Kashi kranti	(10) JDNO 11-12	(11) AOL 13-133	(12) JOL 09-05
(13) AOL 14-11	(14) JOL 13-05	(15) GP OK-296	(16) GP OK-213
(17) GP OK -292	(18) GAO-5	(19) Red okra	(20) Pusa sawani
(21) Parbhani kranti	(22) AOL 13-73	(23) AOL 12-55	(24) AOL 13-88
(25) AOL 13-112	(26) AOL 14-08	(27) AOL 13-90	(28) AOL 12-59
(29) JOL 13-07	(30) AOL 13-144		

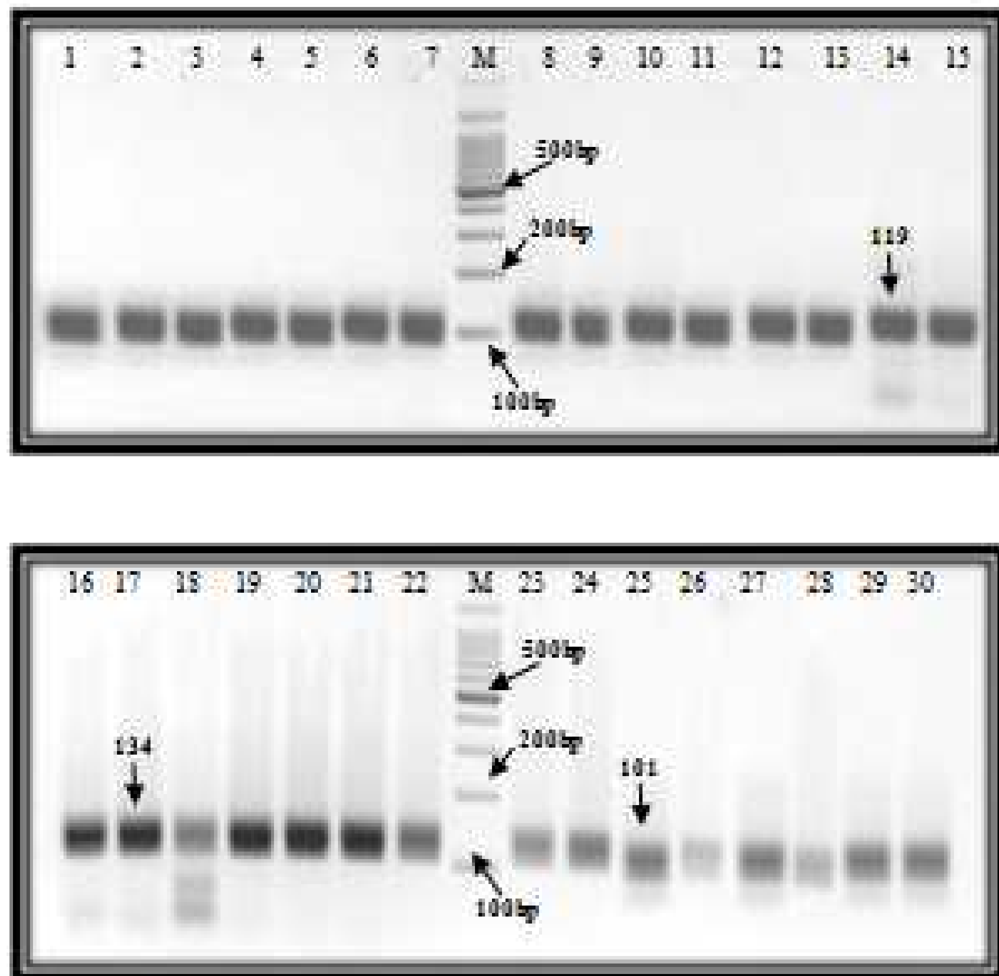


Plate 4.18: SSR profile of OK-2

M = DNA marker

(1) AOL 12-52	(2) AOL 14-32	(3) AOL 13-94	(4) AOL 09-02
(5) AOL 14-29	(6) JOL 11-12	(7) AOL 13-141	(8) JDNO 11-11
(9) Kashi kranti	(10) JDNO 11-12	(11) AOL 13-133	(12) JOL 09-05
(13) AOL 14-11	(14) JOL 13-05	(15) GP OK-296	(16) GP OK-213
(17) GP OK -292	(18) GAO-5	(19) Red okra	(20) Pusa sawani
(21) Parbhani kranti	(22) AOL 13-73	(23) AOL 12-55	(24) AOL 13-88
(25) AOL 13-112	(26) AOL 14-08	(27) AOL 13-90	(28) AOL 12-59
(29) JOL 13-07	(30) AOL 13-144		

4.5.2.1.3 OK-3

The primer OK-3 produced a total of four alleles ranging in size from 117 bp to 138 bp (Plate: 4.19). These four alleles were designated as 'A', 'B', 'C' and 'D'. Out of these four alleles, allele 'A' recorded the highest allele frequency while allele 'B','C' allele 'D' exhibited lowest allele frequency (Table 4.13). Allele 'A'(136 bp) was observed in genotypes *viz.,* AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 and AOL 13-141, JDNO 11-11, Kashi Kranti; allele 'B' (117 bp) in genotypes *viz.*, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296,; allele 'C' (129 bp) in JDNO 11-12, AOL 13-133, GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73 while allele 'D' (138 bp) was observed in genotypes *viz.*, AOL 12-55, AOL 13-88, AOL 13-112, AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 and AOL 13-144. The PIC value for this marker was found to be 0.42. The expected heterozygosity in the present marker was 0.731 (Table 4.12)

Table 4.12: Amplified bands, range of molecular weight, total no. of alleles, PIC, Na, Ne & He value of SSR primers

Sr. No	Locus name	No. Of bands amplified	Molecular weight range (bp)	Total no. Alleles	PIC	Na	Ne	He
1	OK-1	30	212 bp-226 bp	3	0.42	3.00	1.60	0.624
2	OK-2	30	101 bp-134 bp	3	0.44	3.00	1.61	0.620
3	OK-3	30	117 bp-138 bp	4	0.42	4.00	1.37	0.731
4	OK-4	50	158 bp-243 bp	7	0.63	7.00	1.13	0.886
5	OK-5	37	110 bp-180 bp	6	0.62	6.00	1.46	0.684
6	OK-6	30	150 bp-169 bp	3	0.44	3.00	2.10	0.477
7	OK-7	32	105 bp-161 bp	7	0.67	7.00	1.26	0.796
8	OK-8	46	102 bp-209 bp	8	0.64	8.00	1.08	0.928
9	OK-9	59	130 bp-180 bp	4	0.62	4.00	1.42	0.706
10	OK-10	48	103 bp-156 bp	7	0.49	7.0	1.29	0.778
11	OK-11	43	109 bp-147 bp	3	0.42	3.00	1.50	0.665
12	OK-12	30	121 bp-136 bp	3	0.48	3.00	1.59	0.628
13	OK-13	30	131 bp-172 bp	4	0.50	4.00	1.75	0.571
14	OK-14	30	137 bp-167 bp	3	0.50	3.00	1.55	0.650
15	OK-15	52	107 bp-216 bp	6	0.42	6.00	1.24	0.805
Total		**577**	**-**	**71**	**7.71**	**71**	**21.95**	**10.54**
Avg.		**38.46**	**125bp-175bp**	**4.73**	**0.514**	**4.73**	**1.46**	**0.703**

Na = No. of different Alleles,

Ne = No. of effective Alleles,

He = Expected heterozygosity

PIC = Polymorphic Information Content

Table 4.13: Allele description of SSR analysis

Sr. No	Locus name	Allele designation	Allele length (bp)	Allele frequency
1	OK-1	A	223	0.500
		B	212	0.267
		D	226	0.233
2	OK-2	A	119	0.500
		B	134	0.300
		C	101	0.200
3	OK-3	A	136	0.300
		B	117	0.133
		C	138	0.267
		D	129	0.300
4	OK-4	A	243	0.137
		B	205	0.180
		C	223	0.080
		D	190	0.060
		E	158	0.019
		F	234	0.230
		G	199	0.260
5	OK-5	A	141	0.027
		B	174	0.054
		C	180	0.216
		D	145	0.054
		E	149	0.459
		F	110	0.228
6	OK-6	A	157	0.666
		B	169	0.264
		C	150	0.100
7	OK-7	A	105	0.031
		B	161	0.031
		C	145	0.031
		D	137	0.312
		E	133	0.156
		F	128	0.281
		G	110	0.156

8	OK-8	A	209	0.022
		B	165	0.087
		C	102	0.043
		E	171	0.239
		F	159	0.065
		G	127	0.022
		H	145	0.304
		I	182	0.218
9	OK-9	A	180	0.218
		B	149	0.356
		C	153	0.373
		D	130	0.119
10	OK-10	A	140	0.312
		C	119	0.312
		C	118	0.063
		D	103	0.020
		E	127	0.145
		F	156	0.043
		G	107	0.098
11	OK-11	A	121	0.349
		B	147	0.302
		C	109	0.349
12	OK-12	A	125	0.462
		B	121	0.362
		C	136	0.166
13	OK-13	A	166	0.600
		B	131	0.067
		C	172	0.233
		D	152	0.100
14	OK-14	A	137	0.234
		B	152	0.433
		C	167	0.333
15	OK-15	A	216	0.135
		B	142	0.211
		C	120	0.076
		E	168	0.212
		F	167	0.269
		G	107	0.097

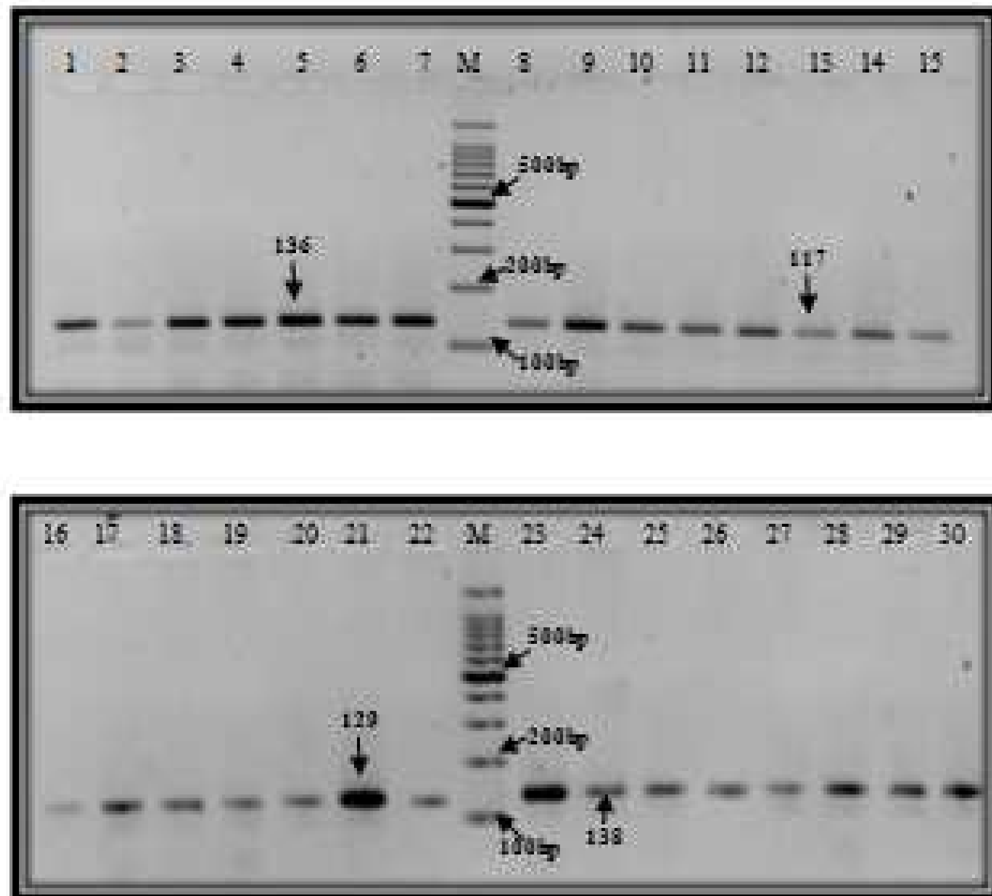


Plate 4.19: SSR profile of OK-3

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-215
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani kranti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

4.5.2.1.4 OK-4

The primer OK-4 produced a total of seven alleles ranging in size from 158 bp to 243 bp (Plate: 4.20). These seven alleles were designated as 'A', 'B', 'C,''D', 'E', 'F' and 'G'. Among seven alleles, allele 'F' recorded the highest allele frequency than allele 'E' and rest of all alleles (Table 4.13). Most unique seven alleles among that allele 'A' was observed in AOL 12-52,AOL 13-141 allele 'B' in JOL 09-05, allele 'C' in JDNO 11-12 and allele 'D','E','F','G' was observed in AOL 14-11, JOL 13-05, GP OK-296,GP OK-123,AOL 13-112, respectively. In some genotypes both pair of alleles are present like alleles 'A' & 'B' was observed in AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12; alleles 'E' &'D' was observed in GP-OK-296 and alleles 'F' &'G' was in GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55, AOL 13-88, AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 and AOL 13-144. The PIC value for this marker was found to be 0.63. The expected heterozygosity in the present marker was 0.886. (Table 4.12)

4.5.2.1.5 OK-5

The primer OK-5 produced a total of six alleles ranging in size from 110 bp to 180 bp (Plate: 4.21). These six alleles were designated as 'A', 'B', 'C' 'D', 'E', and 'F'. Out of these six alleles, allele 'E' recorded the highest allele frequency while in alleles 'A' lowest allele frequency was observed (Table 4.13). Allele 'A'(141 bp) was observed in only genotypes AOL 14-32 ; allele 'B' (174 bp) was observed in genotypes *viz.*, Kashi Kranti,; allele 'C' (180 bp) was observed in genotypes *viz.*, Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL

12-55,; allele 'D' (145 bp) was observed in genotypes *viz*., AOL 12-59; and allele 'E' (149 bp) was observed in genotypes *viz*., AOL 12-52, AOL 13-94, JDNO 11-12, AOL 13-133, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296,JDNO 11-11, AOL 13-88, AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 .In genotypes AOL 09-02,AOL 14-29,AOL 13-141 both the alleles 'E'&'F'(110 bp) are present same allele 'F' along with allele 'B', 'C', 'D' in genotypes JOL 11-12,GP OK-213,GP OK-292,Red Okra, AOL 13-144 respectively. The PIC value for this marker was found to be 0.62. The expected heterozygosity in the present marker was 0.684, respectively (Table 4.12).

4.5.2.1.6 OK-6

The primer OK-6 produced a total of three alleles ranging in size from 150 bp to 169 bp (Plate: 4.22). These three alleles were designated as 'A', 'B', and 'C'. Out of these three alleles, allele 'A' recorded the highest allele frequency while allele 'C' and allele 'B' were lowest allele frequency (Table 4.13). Allele 'A' (157 bp) was observed in genotypes *viz*., AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55, AOL 13-88, AOL 13-112, AOL 14-08, AOL 13-90; allele 'B' (169 bp) was observed in genotypes *viz*., AOL 09-02, AOL 14-29, JOL 11-12 , AOL 13-141, JDNO 11-11, Kashi Kranti, JDNO 11-12 ,AOL 13-133 and JOL 09-05 while allele 'C' (150 bp) was observed in genotypes *viz*., AOL 12-59, AOL JOL 13-07 and AOL 13-144. The PIC value for this marker was found to be 0.44. The expected heterozygosity in the present marker was 0.477 (Table 4.12).

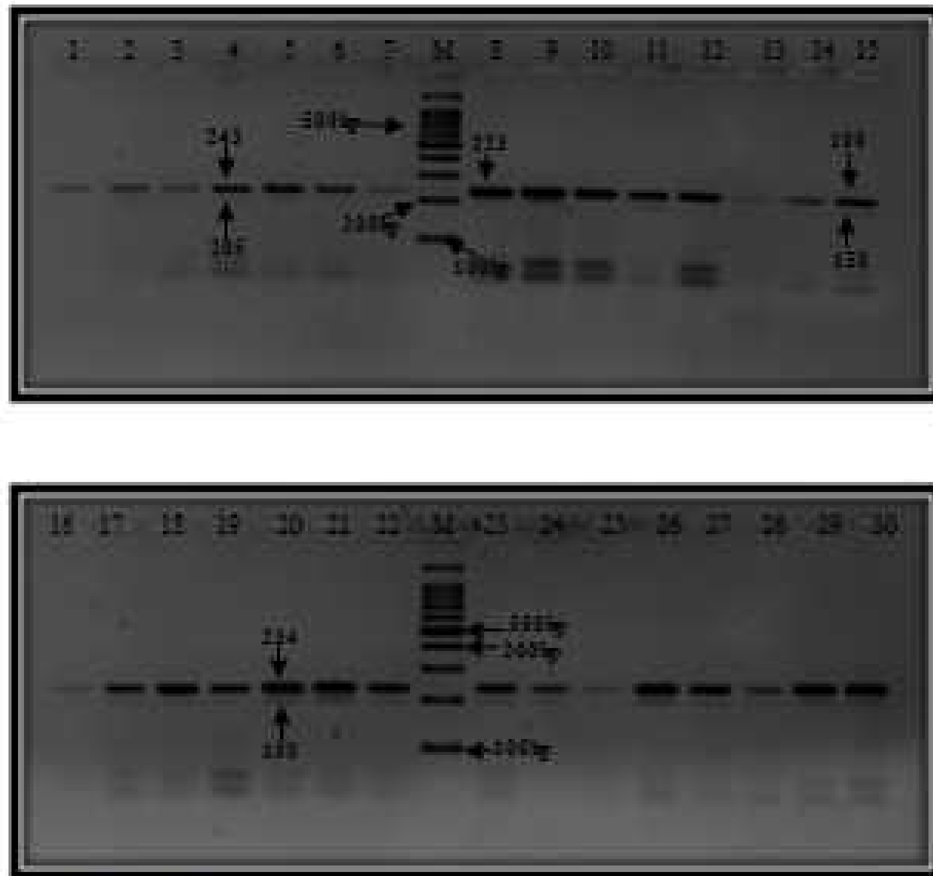


Plate 4.20: SSR profile of OK-4

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani kranti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

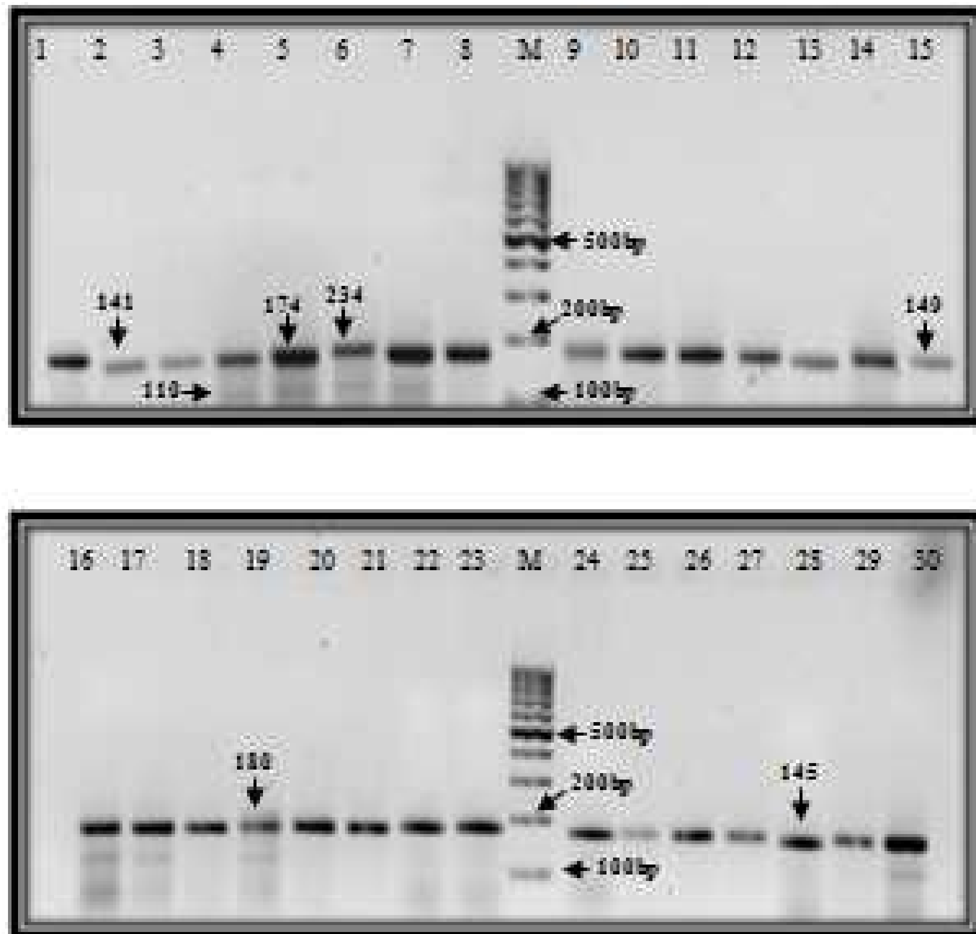
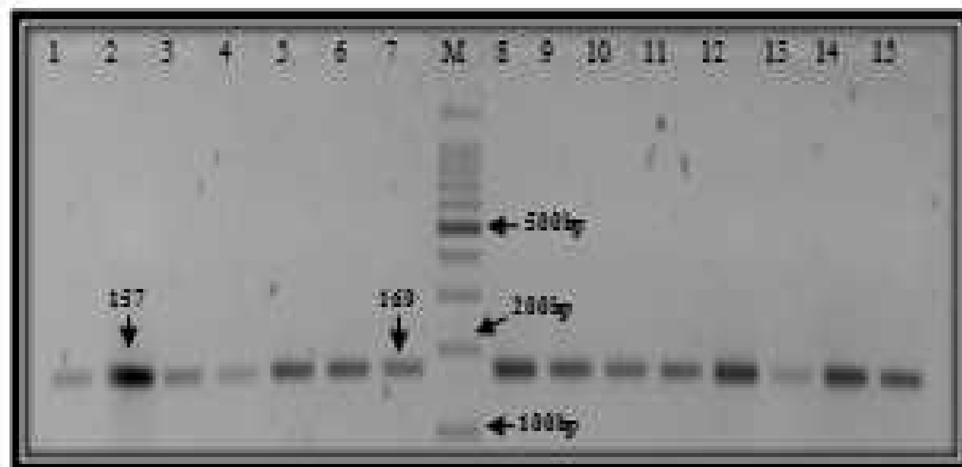


Plate 4.21: SSR profile of OK-5

M = DNA marker

(1) AOL 12-52	(2) AOL 14-32	(3) AOL 13-94	(4) AOL 09-02
(5) AOL 14-29	(6) JOL 11-12	(7) AOL 13-141	(8) JDNO 11-11
(9) Kashi kranti	(10) JDNO 11-12	(11) AOL 13-133	(12) JOL 09-05
(13) AOL 14-11	(14) JOL 13-05	(15) GP OK-296	(16) GP OK-213
(17) GP OK -292	(18) GAO-5	(19) Red okra	(20) Pusa sawani
(21) Parbhani kranti	(22) AOL 13-73	(23) AOL 12-55	(24) AOL 13-88
(25) AOL 13-112	(26) AOL 14-08	(27) AOL 13-90	(28) AOL 12-59
(29) JOL 13-07	(30) AOL 13-144		



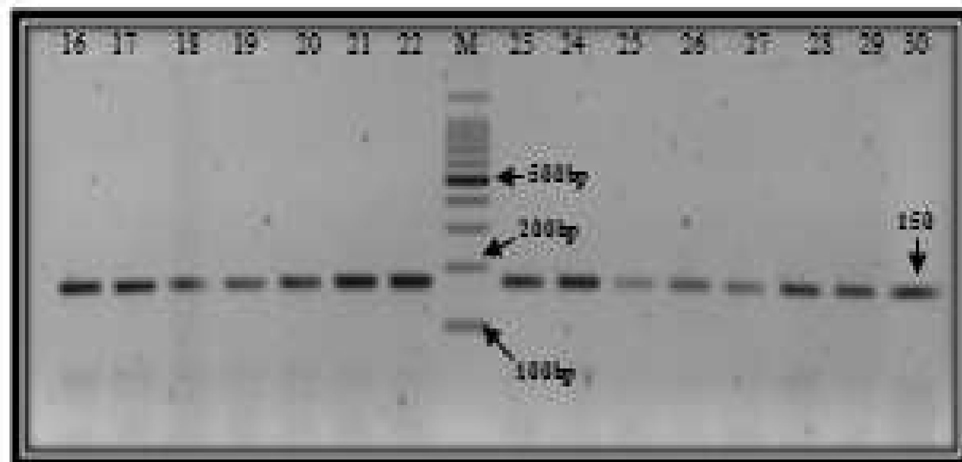


Plate 4.22: SSR profile of OK-6

M = DNA marker

(1) AOL 12-52	(2) AOL 14-32	(3) AOL 13-94	(4) AOL 09-02
(5) AOL 14-29	(6) JOL 11-12	(7) AOL 13-141	(8) JDNO 11-11
(9) Kashi kranti	(10) JDNO 11-12	(11) AOL 13-133	(12) JOL 09-05
(13) AOL 14-11	(14) JOL 13-05	(15) GP OK-296	(16) GP OK-215
(17) GP OK -292	(18) GAO-5	(19) Red okra	(20) Pusa sawani
(21) Parbhani kranti	(22) AOL 13-73	(23) AOL 12-55	(24) AOL 13-88
(25) AOL 13-112	(26) AOL 14-08	(27) AOL 13-90	(28) AOL 12-59
(29) JOL 13-07	(30) AOL 13-144		

4.5.2.1.7 OK-7

The primer OK-7 produced a total of seven alleles ranging in size from 105 bp to 161 bp (Plate: 4.23). These seven alleles were designated as 'A', 'B', 'C', 'D', 'E', 'F' and 'G'. Out of these seven alleles, allele 'D' recorded the highest allele frequency while allele 'A', 'B' and allele 'C' were lowest in allele frequencies (Table 4.13). Allele 'A' (105 bp) was observed in only genotype *viz*., AOL 14-08; allele 'D' (137 bp) was observed in genotypes *viz*., JDNO 11-11, Kashi Kranti, JDNO 11-12, AOL 13-133, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296, GP OK-292, JOL 11-12; allele 'E' (133 bp) was observed in genotypes *viz*.,AOL12-52, AOL14-32, AOL13-94, AOL 09-02, AOL13-141; allele 'F' (128 bp) was observed in genotypes *viz*., Parbhani Kranti ,AOL 13-73, Pusa Sawani, GAO-5, GP OK-213, AOL 13-90,AOL 12-55; allele 'G' (110 bp) was observed in genotypes *viz*., AOL 13-112, AOL 12-59, JOL 13-07 and AOL 13-144.While in genotypes Red Okra, AOL 13-88, GP OK-213 alleles pair 'F'&'G', 'B','C' respectively. The PIC value for this marker was found to be 0.67. The expected heterozygosity in the present marker was 0.796 (Table 4.12).

4.5.2.1.8 OK-8

The primer OK-8 produced a total of eight alleles ranging in size from 102 bp to 209 bp (Plate: 4.24). These eight alleles were designated as 'A', 'B', 'C', 'D', 'E', 'F', 'G', and 'H'. Out of these eight alleles, allele 'H' recorded the highest allele frequency and allele 'A'&'G' the lowest (Table 4.13). Alleles 'A', 'B', 'C', 'G' present in AOL 12-52 ; alleles 'D', 'C', 'G' present in JDNO 11-

11;alleles ‘H’,‘G’, present in, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55, AOL 13-88, AOL 14-08, AOL 13-90 , AOL 12-59; allele ‘F’,‘E’, present in GP OK-213; while allele ‘D’,‘G’, present in JOL 13-07 and JOL 13-144; allele ‘D’ was observed in other genotypes *viz*., JOL -11-12, AOL 13-141, JDNO 11-12, AOL-13-133, JOL 13-05, GP OK-296, GP OK-292; allele ‘E’ was observed in GP OK-296 ,and Red okra. The PIC value for this marker was found to be 0.64. The expected heterozygosity in the present marker was 0.928 (Table 4.12).

4.5.2.9 OK-9

The primer OK-9 produced a total of four alleles ranging in size from 130 bp to 180 bp (Plate: 4.25). These four alleles were designated as ‘A’, ‘B’,‘C’ and ‘D’. Among four alleles, allele ‘B’ recorded the highest allele frequency then allele ‘A’ (Table 4.13). Allele pair ‘A’(180 bp) & ‘B’(149 bp) was observed in genotypes *viz.,* AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 and AOL 13-141, JDNO 11-11,JDNO 11-12, AOL 13-133, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296, GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55, AOL 13-88; only ‘B’(149 bp) was observed in genotype in Kashi Kranti; allele Pair ‘C’(153 bp) & ‘D’(130 bp) was observed in genotypes *viz*., AOL 13-112, AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 and AOL 13-144 ; while allele Pair ‘C’(153 bp) & ‘A’(180 bp) was observed in genotypes *viz*., AOL 12-55,AOL 13-88. The PIC value for this marker was found to be 0.62.The expected heterozygosity in the present marker was 0.706 (Table 4.12).

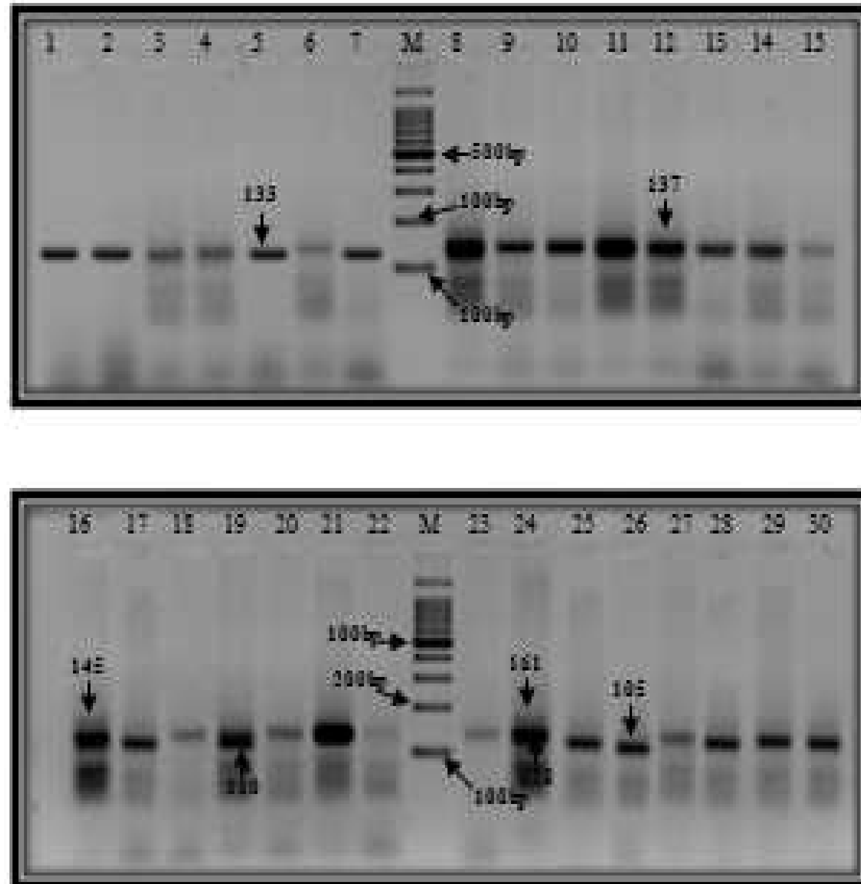


Plate 4.23: SSR profile of OK-7

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani kranti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

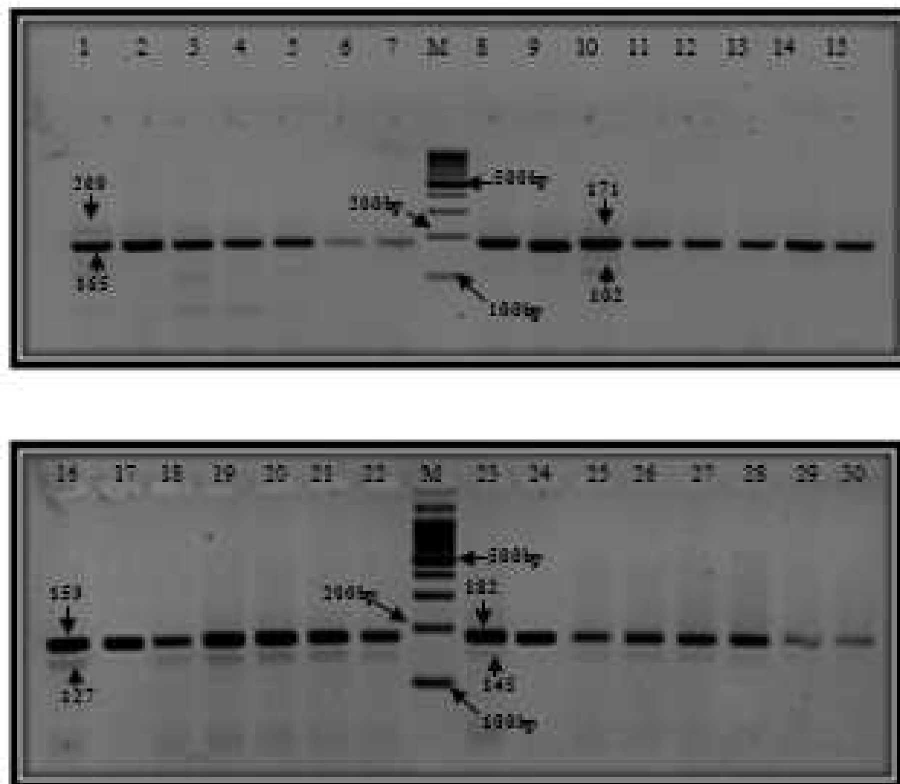


Plate 4.24: SSR profile of OK-8

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani kranti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

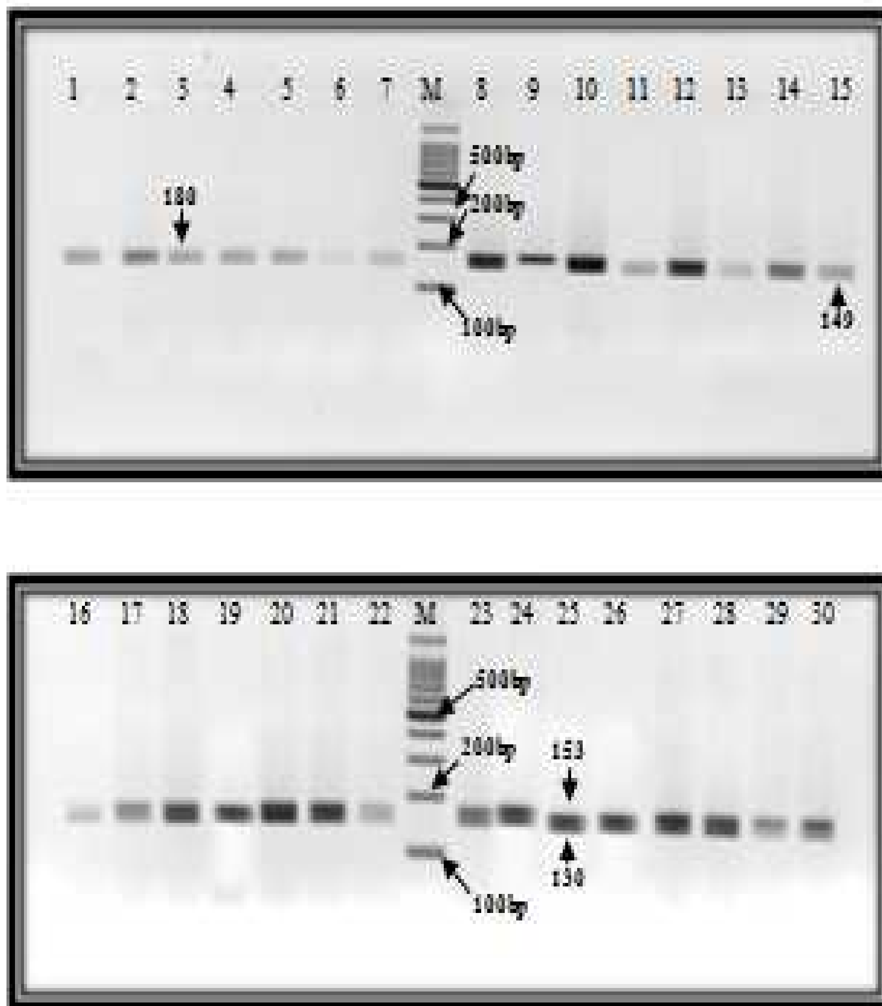


Plate 4.25: SSR profile of OK-9

M = DNA marker

(1) AOL 12-52 (2) AOL 14-52 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani kranti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

4.5.2.1.10 OK-10

The primer OK-10 produced a total of seven alleles ranging in size from 103 bp to 156 bp (Plate: 4.26). These seven alleles were designated as 'A', 'B', 'C', 'D', 'E', 'F' and 'G'. Out of these seven alleles, allele 'A'&'C' recorded the highest allele frequency while allele 'D' lowest allele frequency (Table 4.13). allele pair 'A',& 'B' was observed in genotypes *viz.,* AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 and AOL 13-141, JDNO 11-11,JDNO 11-12, AOL 13-133, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296; allele pair 'E',& 'F' was observed in genotypes *viz.,* GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73; allele 'E' was observed in AOL 13-112, AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 .While only allele 'E' was observed in AOL 13-144. The PIC value for this marker was found to be 0.49.The expected heterozygosity in the present marker was 0.778 (Table 4.12).

4.5.2.1.11 OK-11

The primer OK-11 produced a total of three alleles ranging in size from 109 bp to 147 bp (Plate: 4.27). These three alleles were designated as 'A', 'B', and 'C'. Out of these three alleles, allele 'A', and 'C' recorded the highest allele frequency while allele 'B' the lowest (Table 4.13). Allele pair 'A' &'C' was observed in genotypes *viz.,* JDNO 11-11,Kashi Kranti ,JDNO 11-12, AOL 13-133, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296; allele pair 'B' &'C' was observed in genotypes *viz.*, GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55, AOL 13-88, AOL 13-112,

AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 and AOL 13-144. while allele 'A' was observed in genotypes *viz*., AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 and AOL 13-141. The PIC value for this marker was found to be 0.42. The expected heterozygosity in the present marker was 0.665 (Table 4.12).

4.5.2.1.12 OK-12

The primer OK-12 produced only a three allele of a size 121-136 bp (Plate: 4.28). The allele was designated as 'A''B' and 'C'. The allele frequency of allele 'A' is higher allele frequency 'C'. Allele 'A' (125 bp) was observed in all genotypes *viz.,* AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 ,AOL 13-141, JDNO 11-11,JDNO 11-12, AOL 13-133, JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296; Allele 'B' (136 bp) was observed in all genotypes *viz.,* Kashi Kranti , Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55; While allele 'C' (121 bp) was observed in all genotypes *viz.,* GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73, AOL 12-55, AOL 13-88, AOL 13-112, AOL 14-08, AOL 13-90 , AOL 12-59, JOL 13-07 and AOL 13-144. The PIC value and expected heterozygosity for this marker was found to be 0.48 and 0.628, respectively (Table: 4.12).

4.5.2.1.13 OK-13

The primer OK-13 produced a total of four alleles ranging in size from 131 to 172 bp (Plate: 4.29). These four alleles were designated as 'A','B' 'C' and 'D'. Among four alleles, allele 'A' recorded the highest allele frequency then allele 'B' (Table 4.13). Allele 'A' (166 bp) was observed in seventeen genotypes

i.e. AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 ,AOL 13-141, JDNO 11-11, Kashi Kranti , JDNO 11-12, AOL 13-133, AOL 12-55, AOL 13-88, AOL 13-112, AOL 14-08, AOL 13-90 , AOL 12-59; allele 'B' (131 bp) was observed in two genotypes i.e. JOL 13-07, AOL 13-144; allele 'C' (172 bp) was observed in seven genotypes i.e. GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73; allele 'C' (172 bp) was observed in three genotypes i.e. JOL 09-05, AOL 14-11, JOL 13-05.The PIC value and expected heterozygosity for this marker was found to be 0.50 and 0.571, respectively (Table: 4.12).

4.5.2.1.14 OK-14

The primer OK-14 produced a total of three alleles ranging in size from 131 to bp (Plate: 4.30). These three alleles were designated as 'A', 'B', and 'C'. Out of these four alleles, allele 'B' recorded the highest allele frequency while allele 'A' lowest allele frequency (Table 4.13). Allele 'A' (137 bp) was observed in only seven genotypes i.e. AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 ,AOL 13-141; allele 'B' (152 bp) was observed in thirteen genotypes i.e. JDNO 11-11, Kashi Kranti , JDNO 11-12, AOL 13-133, AOL 14-08, AOL 13-90 , JOL 09-05, AOL 14-11, JOL 13-05, GP OK-296, AOL 12-59,JOL 13-07 and AOL 13-144; while allele 'C' (167 bp) was observed in ten genotypes i.e. AOL 13-112, AOL 12-55, GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73 and AOL 13-88. The PIC value for this marker was found to be 0.50. The expected heterozygosity in the present marker was 0.650 (Table 4.12).

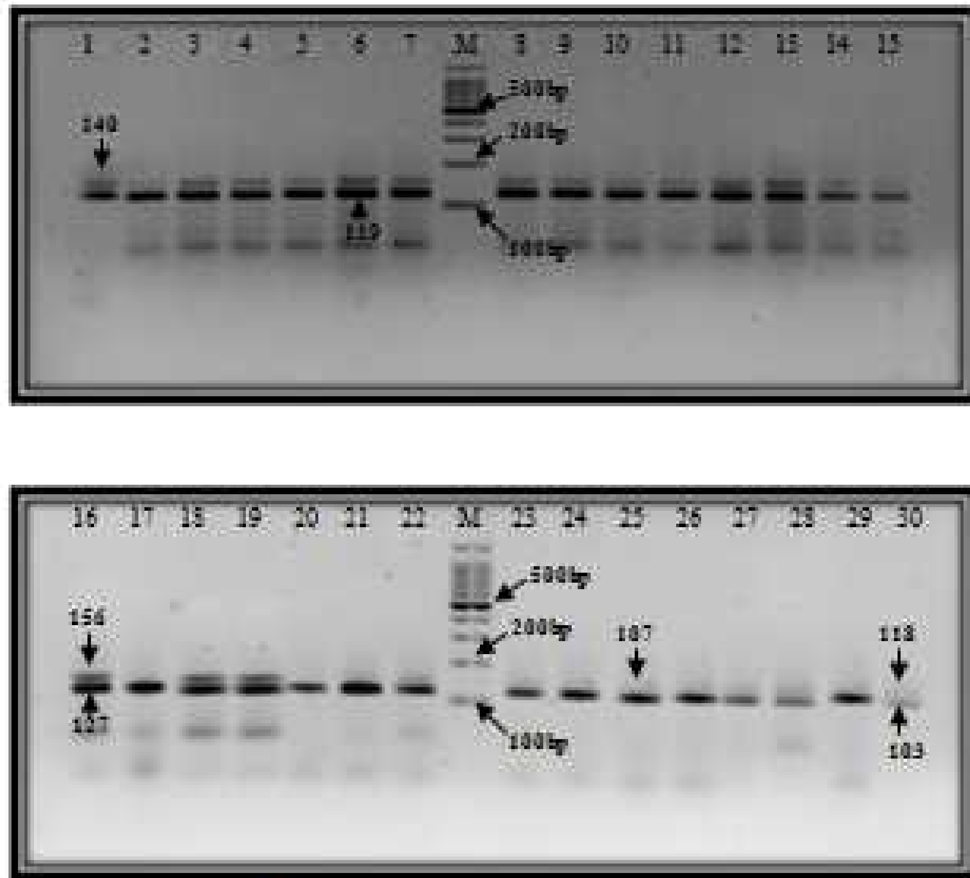


Plate 4.26: SSR profile of OK-10

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-03 (15) GP OK-296 (16) GP OK-215
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

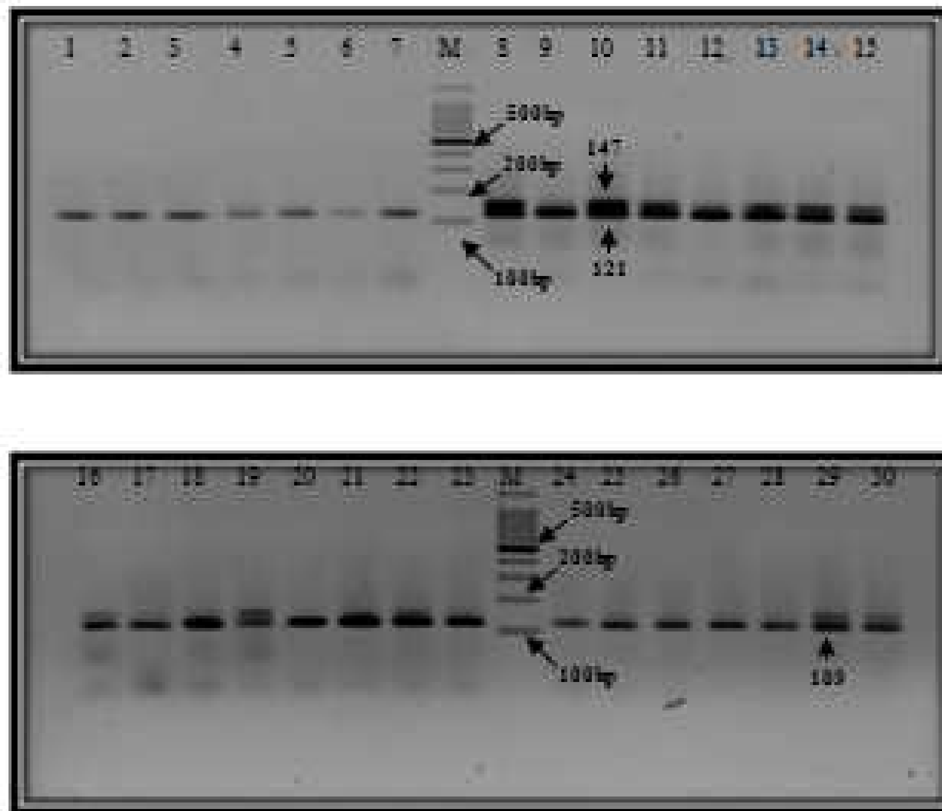


Plate 4.27: SSR profile of OK-11

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-135 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-215
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani kranti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

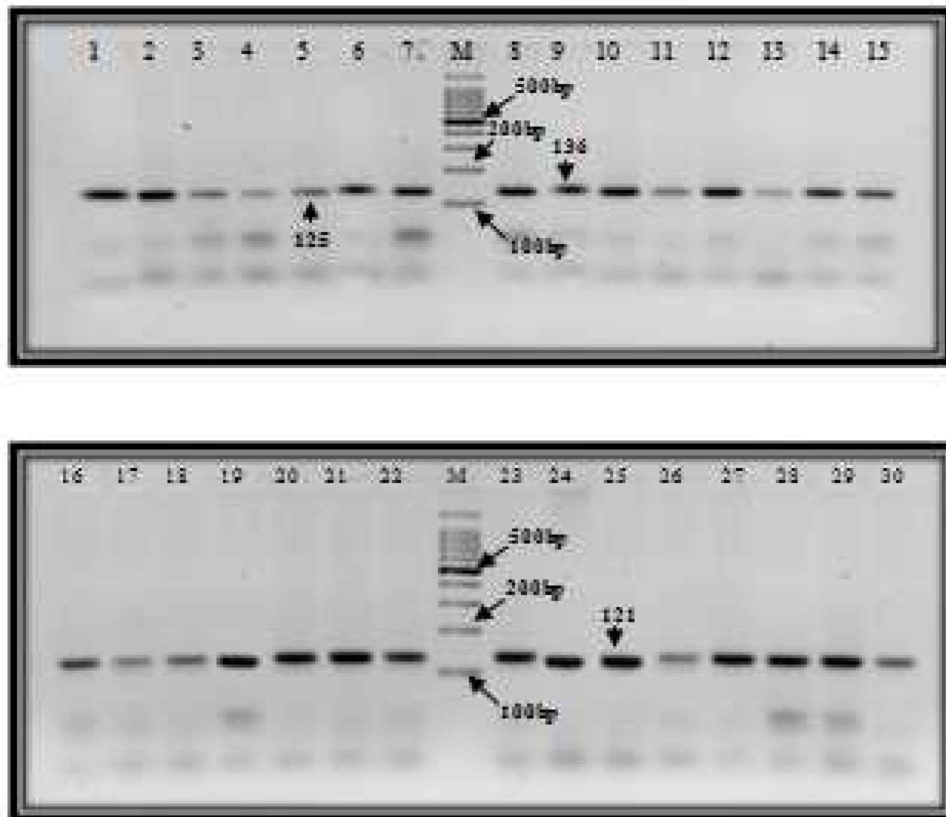


Plate 4.28: SSR profile of OK-12

M = DNA marker

(1) AOL 12-52 (2) AOL 14-52 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

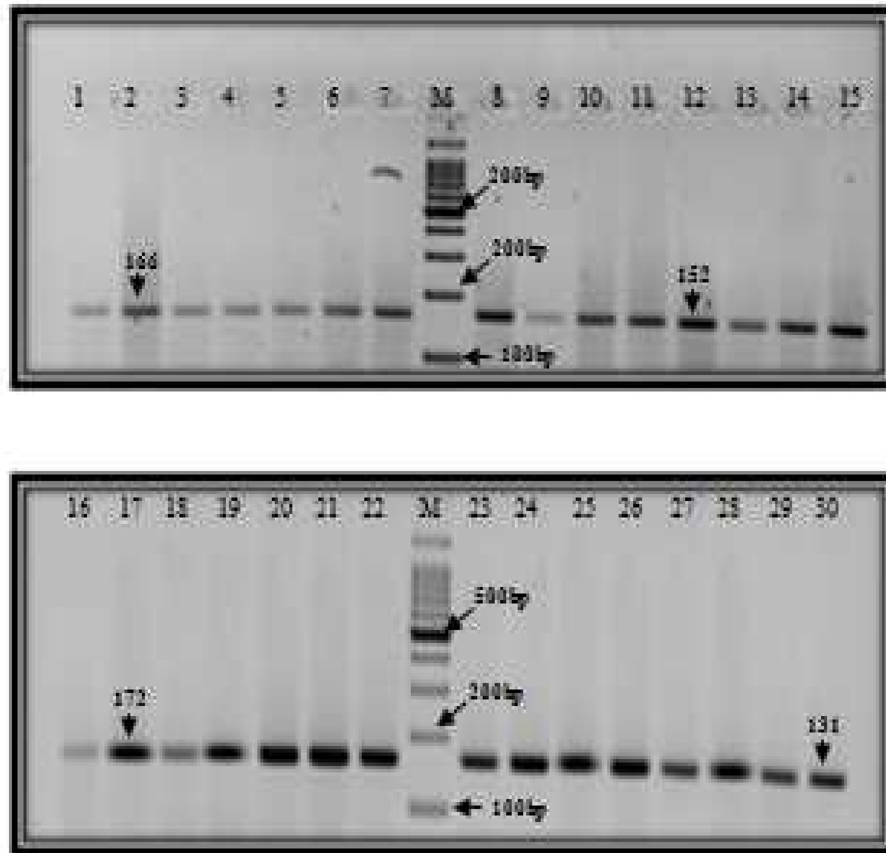


Plate 4.29: SSR profile of OK-13

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani kranti (22) AOL 13-73 (23) AOL 12-53 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

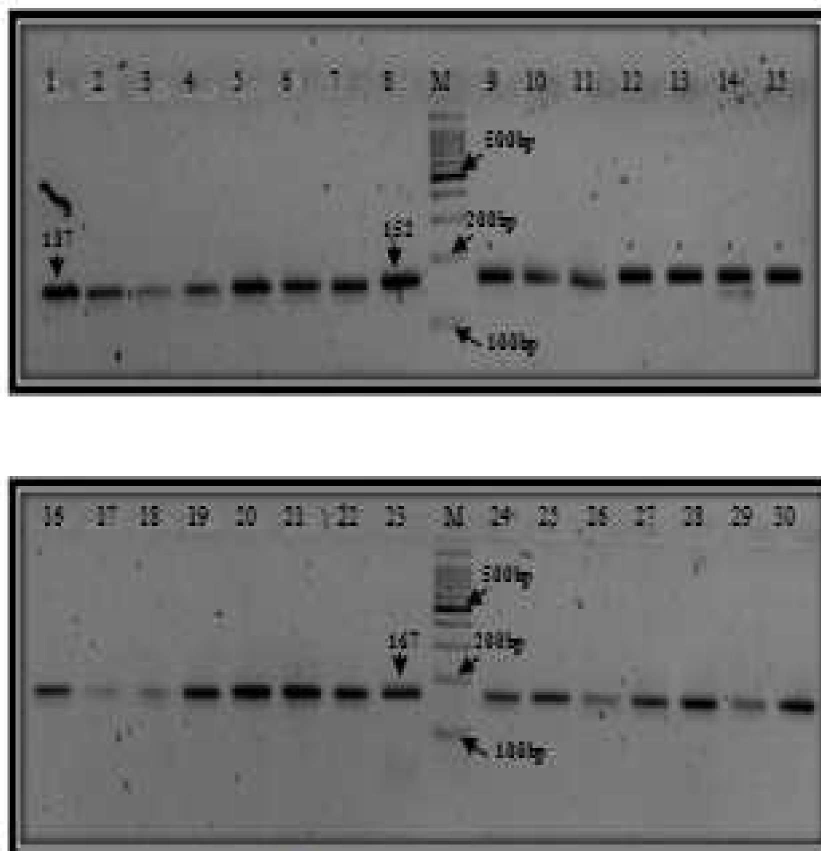


Plate 4.30: SSR profile of OK-14

M = DNA marker

(1) AOL 12-52 (2) AOL 14-32 (3) AOL 13-94 (4) AOL 09-02
(5) AOL 14-29 (6) JOL 11-12 (7) AOL 13-141 (8) JDNO 11-11
(9) Kashi kranti (10) JDNO 11-12 (11) AOL 13-133 (12) JOL 09-05
(13) AOL 14-11 (14) JOL 13-05 (15) GP OK-296 (16) GP OK-213
(17) GP OK -292 (18) GAO-5 (19) Red okra (20) Pusa sawani
(21) Parbhani karnti (22) AOL 13-73 (23) AOL 12-55 (24) AOL 13-88
(25) AOL 13-112 (26) AOL 14-08 (27) AOL 13-90 (28) AOL 12-59
(29) JOL 13-07 (30) AOL 13-144

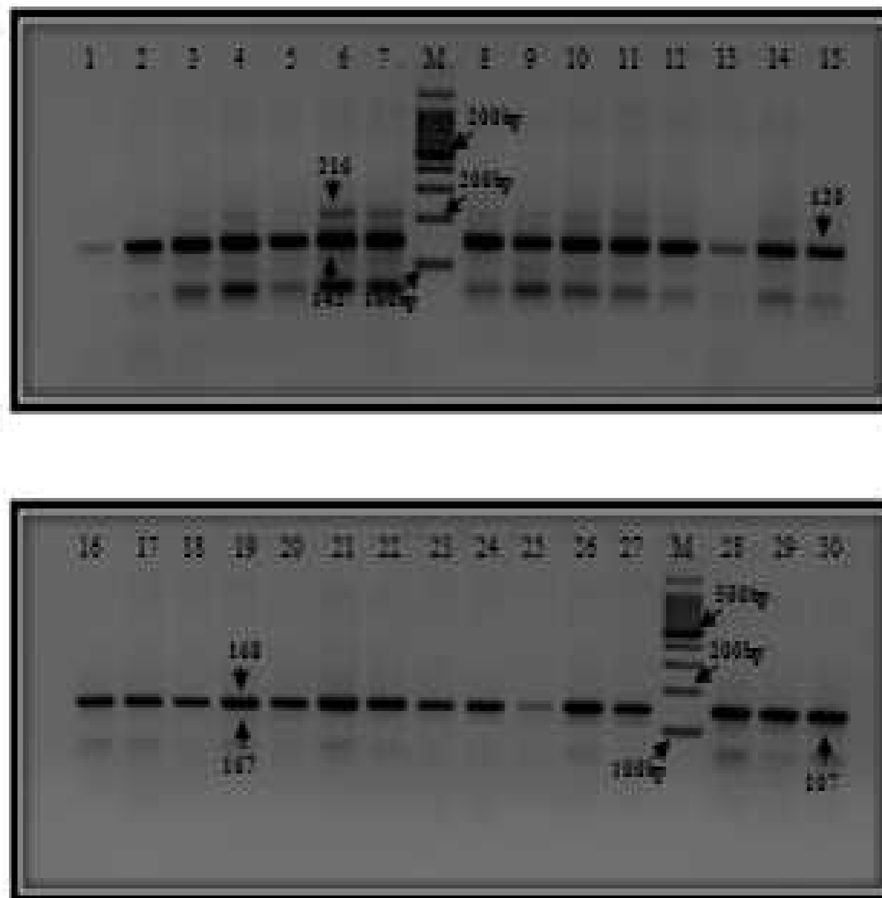


Plate 4.31: SSR profile of OK-15

M = DNA marker

(1) AOL 12-52	(2) AOL 14-32	(3) AOL 13-94	(4) AOL 09-02
(5) AOL 14-29	(6) JOL 11-12	(7) AOL 13-141	(8) JDNO 11-11
(9) Kashi kranti	(10) JDNO 11-12	(11) AOL 13-133	(12) JOL 09-05
(13) AOL 14-11	(14) JOL 13-05	(15) GP OK-296	(16) GP OK-213
(17) GP OK -292	(18) GAO-5	(19) Red okra	(20) Pusa sawani
(21) Parbhani kranti	(22) AOL 13-73	(23) AOL 12-55	(24) AOL 13-88
(25) AOL 13-112	(26) AOL 14-08	(27) AOL 13-90	(28) AOL 12-59
(29) JOL 13-07	(30) AOL 13-144		

4.5.2.1.15 OK-15

The primer OK-15 produced a total of six alleles ranging in size from 120 to 216 bp (Plate: 4.31). These six alleles were designated as 'A', 'B', 'C', 'D' 'E', and 'F'. Out of these six alleles, allele 'F' recorded the highest allele frequency while allele 'C' lowest allele frequency (Table 4.13). Allele 'A' &'B' pair was observed in only six genotypes i.e. JOL 11-12, AOL 13-141, JDNO 11-11, Kashi Kranti, JDNO 11-12 and AOL 13-133; allele 'E'& 'D' pair was observed in genotypes *viz*., GP OK-213, GP OK-292, GAO-5, Red Okra, Pusa Sawani, Parbhani Kranti, AOL 13-73,AOL 13-88,AOL 13-112; allele pair 'E'& 'F' was observed in genotypes *viz*., AOL 13-90 , AOL 12-59,JOL 13-07, AOL 13-144; allele 'B' was observed in genotypes *viz*., AOL 12-52, AOL 14-32, AOL 13-94, AOL 09-02, AOL 14-29; while allele 'C' was observed in genotypes *viz*., AOL 12-52, JOL 13-05 .The PIC value for this marker was found to be 0.42. The expected heterozygosity in the present marker was 0.805 (Table 4.12).

4.5.1.2 Pooled SSR

4.5.1.2.1 Pooled SSR data analysis

In the present SSR analysis with 15 microsatellite markers, all markers gave the results and produced 71 alleles. The average number of alleles per locus was found to be 4.73. The effective number of alleles was 1.46. The maximum number of alleles was eight which were recorded for markers OK-8 followed by OK-4, OK-7 and OK-10 which produced seven alleles. OK-5 and OK-15 produced six alleles. OK-2, OK-3, OK-9 and OK-13 produced four alleles. Whereas OK-1, OK-6, OK-11, OK-12 and OK-14 produced three alleles.

The highest allele frequency found by OK-6 marker was 0.666. The highest PIC value was recorded for OK-08 (0.64), and the lowest for OK-1, OK-3, OK-11 and OK-13 (0.42). The molecular weight of the amplified PCR products ranged from 101bp (OK-2) to 226 bp (OK-1) (Table 4.12). Based on the SSR data, cluster analysis was performed using genetic similarity values and a dendrogram was generated showing genetic relationships among all okra genotypes.

Based on the SSR data, cluster analysis was performed using genetic similarity values and a dendrogram was generated showing the grouping of genotypes according to their genetic diversity. The highest similarity index (Table 4.14) value of 0.95 was found between AOL 13-141 and AOL 13-133 genotypes, while the least similarity index value of 0.15 was found between JOL 13-05 and AOL 14-29. The average similarity coefficient among genotypes was 0.54.

Marker Index values were calculated according to the formulas described in section 3.10.4. Marker index value for pooled SSR data was found to be 5.78. The high value of Marker Index can be justified due to the high value of Effective Multiplex Ratio (EMR) component. EMR value is affected by the number of polymorphic and total number of loci amplified. Based on the SSR data, cluster analysis was performed using genetic similarity values and a dendrogram was generated showing genetic relationship among all okra genotypes.

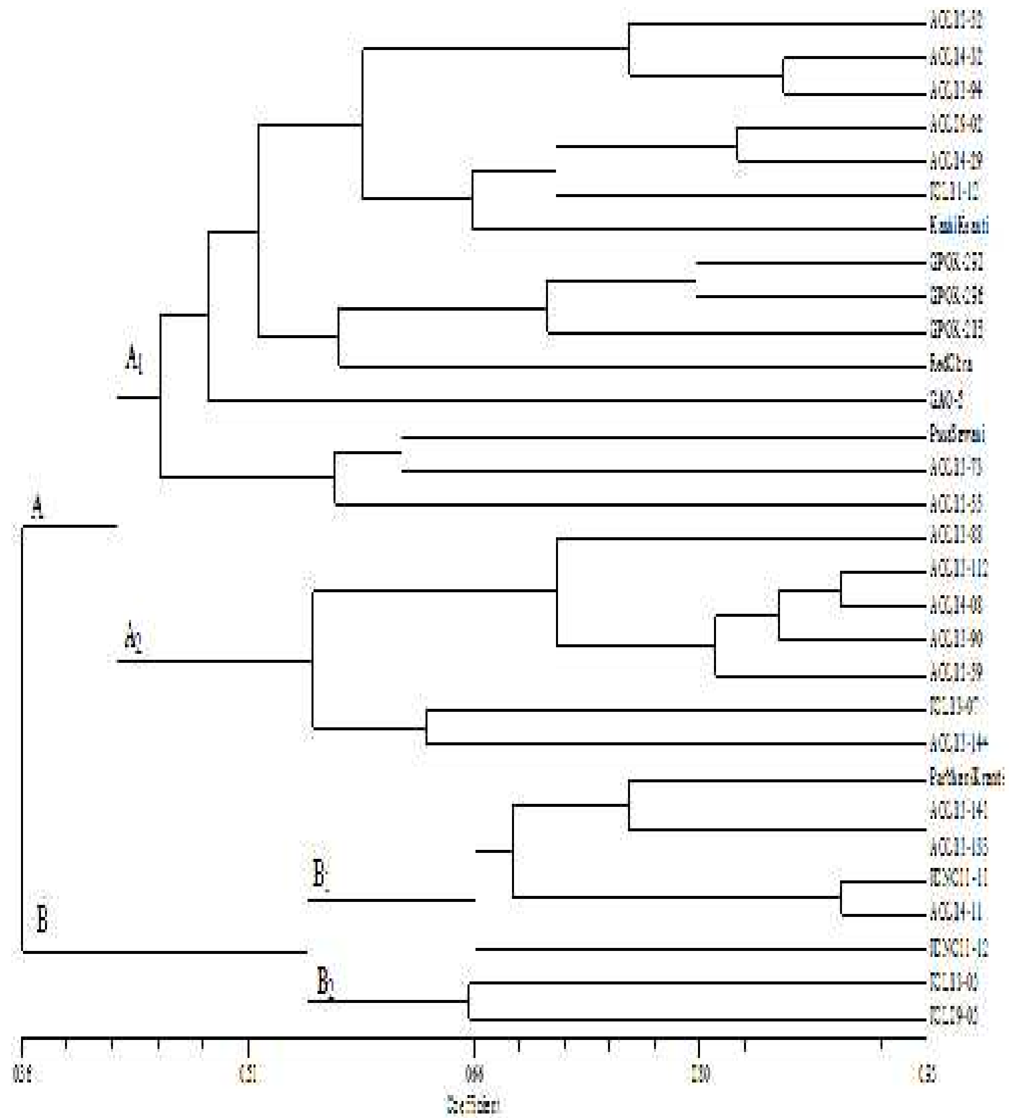


Fig. 4.14: Dendogram showing clustering of thirty okra genotypes constructed using UPGMA based on Jaccard's coefficient obtained from SSR analysis

Table 4.14: Genetic similarity matrix of SSR data based on Jaccard's similarity coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1.00																													
2	0.77	1.00																												
3	0.74	0.66	1.00																											
4	0.52	0.67	0.71	1.00																										
5	0.56	0.67	0.76	0.85	1.00																									
6	0.54	0.65	0.67	0.71	0.71	1.00																								
7	0.39	0.41	0.44	0.67	0.60	0.71	1.00																							
8	0.35	0.39	0.45	0.46	0.52	0.48	0.56	1.00																						
9	0.22	0.27	0.30	0.36	0.36	0.34	0.46	0.77	1.00																					
10	0.35	0.33	0.35	0.45	0.45	0.48	0.56	0.56	0.72	1.00																				
11	0.21	0.26	0.29	0.41	0.57	0.53	0.95	0.74	0.58	0.73	1.00																			
12	0.27	0.33	0.31	0.39	0.50	0.36	0.42	0.55	0.76	0.67	0.82	1.00																		
13	0.31	0.32	0.36	0.34	0.26	0.32	0.37	0.52	0.68	0.60	0.75	0.69	1.00																	
14	0.25	0.20	0.25	0.19	0.15	0.23	0.24	0.42	0.57	0.44	0.34	0.59	0.67	1.00																
15	0.32	0.29	0.37	0.35	0.31	0.28	0.29	0.41	0.34	0.46	0.38	0.63	0.70	0.65	1.00															
16	0.46	0.56	0.54	0.57	0.69	0.48	0.50	0.45	0.45	0.30	0.43	0.37	0.32	0.22	0.38	1.00														
17	0.45	0.52	0.50	0.54	0.65	0.45	0.41	0.50	0.46	0.32	0.45	0.38	0.36	0.22	0.39	0.80	1.00													
18	0.52	0.63	0.52	0.60	0.66	0.47	0.46	0.42	0.39	0.46	0.42	0.32	0.29	0.19	0.37	0.61	0.59	1.00												
19	0.39	0.45	0.46	0.52	0.62	0.44	0.46	0.45	0.45	0.45	0.44	0.38	0.33	0.30	0.47	0.75	0.66	0.56	1.00											
20	0.50	0.56	0.56	0.54	0.64	0.34	0.41	0.30	0.32	0.41	0.34	0.33	0.32	0.16	0.39	0.62	0.59	0.59	0.50	1.00										
21	0.36	0.46	0.46	0.56	0.56	0.32	0.54	0.52	0.37	0.45	0.42	0.39	0.29	0.17	0.18	0.52	0.43	0.50	0.38	0.48	1.00									
22	0.36	0.42	0.41	0.39	0.50	0.42	0.37	0.43	0.48	0.34	0.46	0.38	0.33	0.22	0.26	0.58	0.60	0.53	0.47	0.62	0.53	1.00								
23	0.22	0.27	0.34	0.46	0.45	0.34	0.41	0.39	0.32	0.32	0.36	0.48	0.42	0.33	0.43	0.48	0.57	0.44	0.50	0.52	0.42	0.62	1.00							
24	0.34	0.46	0.50	0.60	0.45	0.44	0.41	0.30	0.32	0.37	0.34	0.37	0.32	0.24	0.38	0.48	0.52	0.48	0.45	0.46	0.42	0.42	0.38	1.00						
25	0.42	0.56	0.46	0.52	0.46	0.48	0.50	0.28	0.29	0.44	0.32	0.35	0.30	0.26	0.42	0.39	0.50	0.42	0.39	0.38	0.50	0.35	0.38	0.72	1.00					
26	0.39	0.41	0.50	0.54	0.48	0.38	0.52	0.30	0.32	0.46	0.34	0.37	0.32	0.29	0.43	0.48	0.57	0.59	0.45	0.42	0.52	0.37	0.32	0.73	0.89	1.00				
27	0.46	0.44	0.54	0.56	0.52	0.34	0.50	0.29	0.30	0.45	0.33	0.36	0.31	0.28	0.42	0.39	0.52	0.47	0.44	0.44	0.42	0.42	0.36	0.77	0.85	0.86	1.00			
28	0.46	0.50	0.60	0.51	0.46	0.48	0.50	0.29	0.30	0.45	0.33	0.36	0.41	0.37	0.52	0.39	0.52	0.50	0.44	0.44	0.52	0.52	0.44	0.69	0.76	0.86	0.82	1.00		
29	0.36	0.46	0.50	0.42	0.57	0.39	0.25	0.24	0.25	0.27	0.24	0.32	0.36	0.43	0.42	0.38	0.32	0.26	0.32	0.28	0.28	0.52	0.38	0.52	0.43	0.52	0.44	0.57	1.00	
30	0.46	0.50	0.54	0.57	0.47	0.48	0.45	0.39	0.40	0.41	0.43	0.49	0.52	0.45	0.60	0.39	0.36	0.59	0.44	0.35	0.52	0.34	0.45	0.56	0.54	0.62	0.58	0.72	0.66	1.00

(1:AOL 12-52, 2:AOL 14-32, 3 AOL 13-94, 4:AOL 09-02, 5:AOL 14-29, 6 JOL 11-11, 7 AOL 13-141, 8 JDNO 11-11, 9 Kashi Kranti, 10 JDNO 11-12, 11: AOL 13-135,12 JOL 09-05, 13 AOL 14-11, 14 JOL 13-05, 15 GP OK-296, 16:GP OK-213, 17:GP OK-292, 18 GAO-5, 19 Red Okra, 20 Pusa Sawani, 21 Parbhani Kranti, 22:AOL 13-75, 23:AOL 12-55, 24:AOL 13-88, 25:AOL 13-112, 26:AOL 14-08, 27 AOL 13-90, 28 AOL 12-59, 29 JOL 13-07 and 30:AOL 13-144)

4.5.1.3 Clustering Pattern of Different SSR Primers used

Clustering pattern of dendrogram generated by using the pooled molecular data of 15 SSR loci indicated that two clusters namely A and B were formed at a similarity coefficient of 0.36 (Fig. 4.14). Cluster A was divided into two sub clusters A_1 and A_2. Grouping of twenty two genotypes that are in one major cluster 'A'. Sub-cluster A1 included okra genotypes *viz*., AOL 12-52, AOL 14-32, AOL 13-94, AOL-09-02, AOL 14-29, JOL 11-12, Kashi Kranti, GP OK-292, GP-OK-213, GP OK-296, Red okra, GAO-5, Pusa Sawani, AOL 13-73 and AOL 12-55. Sub-cluster A2 consists of genotypes AOL 13-112, AOL 14-08, AOL 13-90, AOL 12-59, JOL 13-07, AOL 13-144 and AOL 13-88. Cluster B was divided into two sub-clusters B1 and B2. Grouping of eight genotypes that are one major cluster 'B'. Sub-cluster B1 consists of genotypes *viz*; Parbhani Kranti, AOL 13-141, AOL 13-133, JDNO 11-11, AOL 14-11 and JDNO 11-12; whereas sub-cluster B2 consists of genotypes *viz*., JOL 13-05 and JOL09-05.

4.6.1.4 Principle component analysis (PCA)

The principle component analysis (PCA) results (Fig 4.15 and Fig.4.16) almost coincided with the results of cluster analysis. The clustering pattern of 2D and 3D of PCA analysis were in accordance with the dendrogram clustering pattern which showed that genotypes (Fig 4.14).

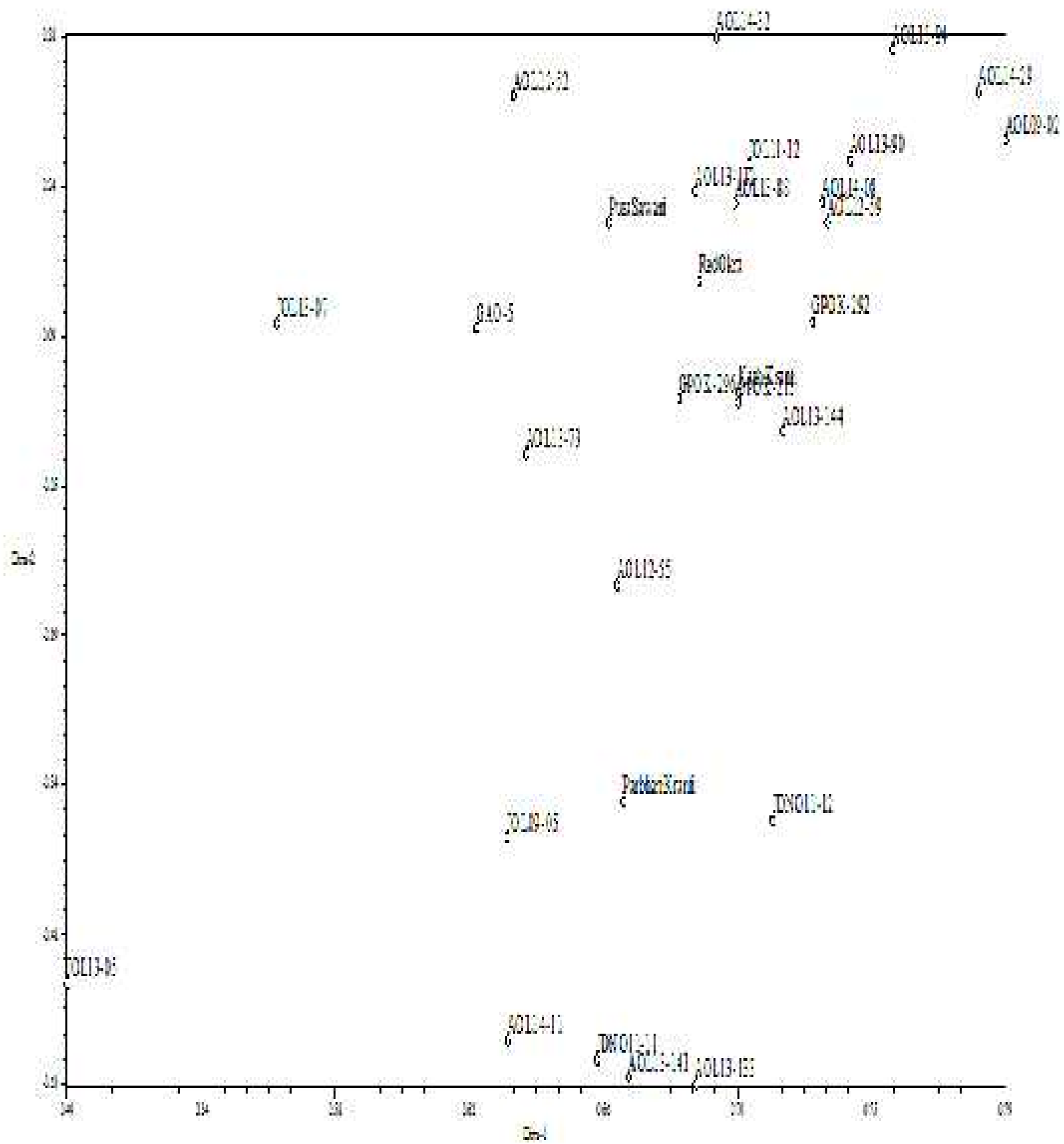


Fig. 4.15: Two dimensional plot of SSR

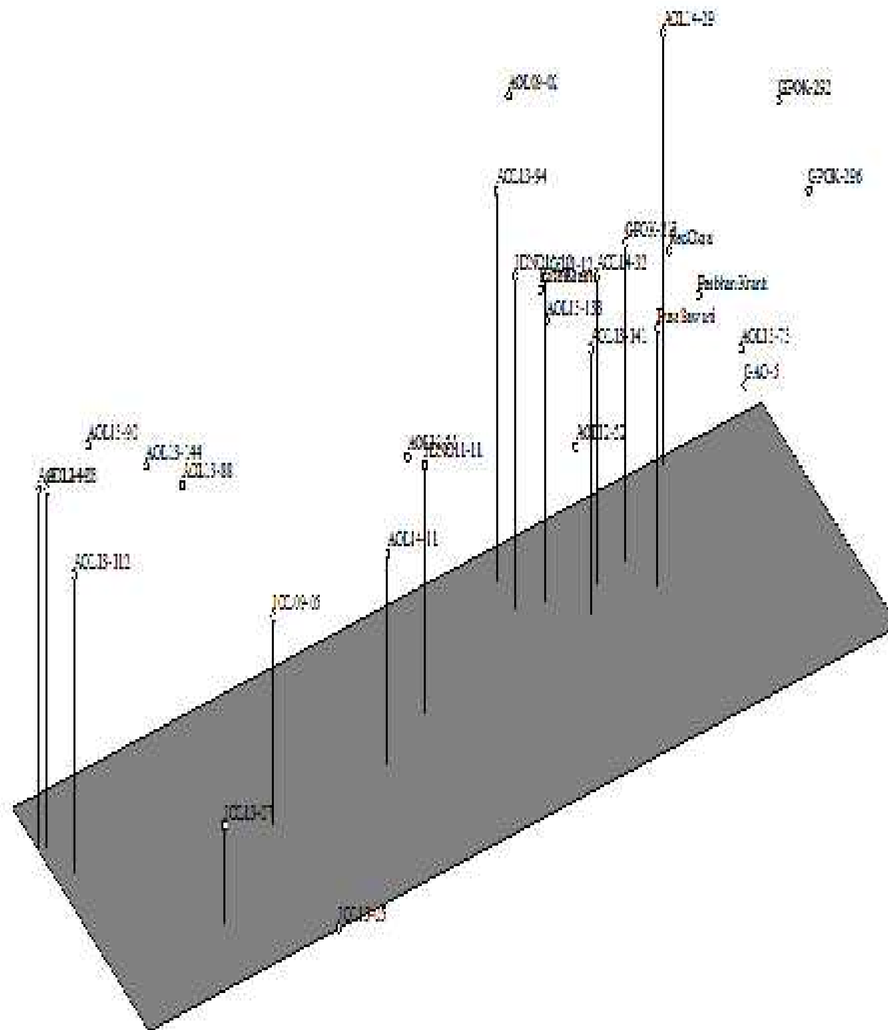


Fig. 4.16: Three dimensional plot of SSR

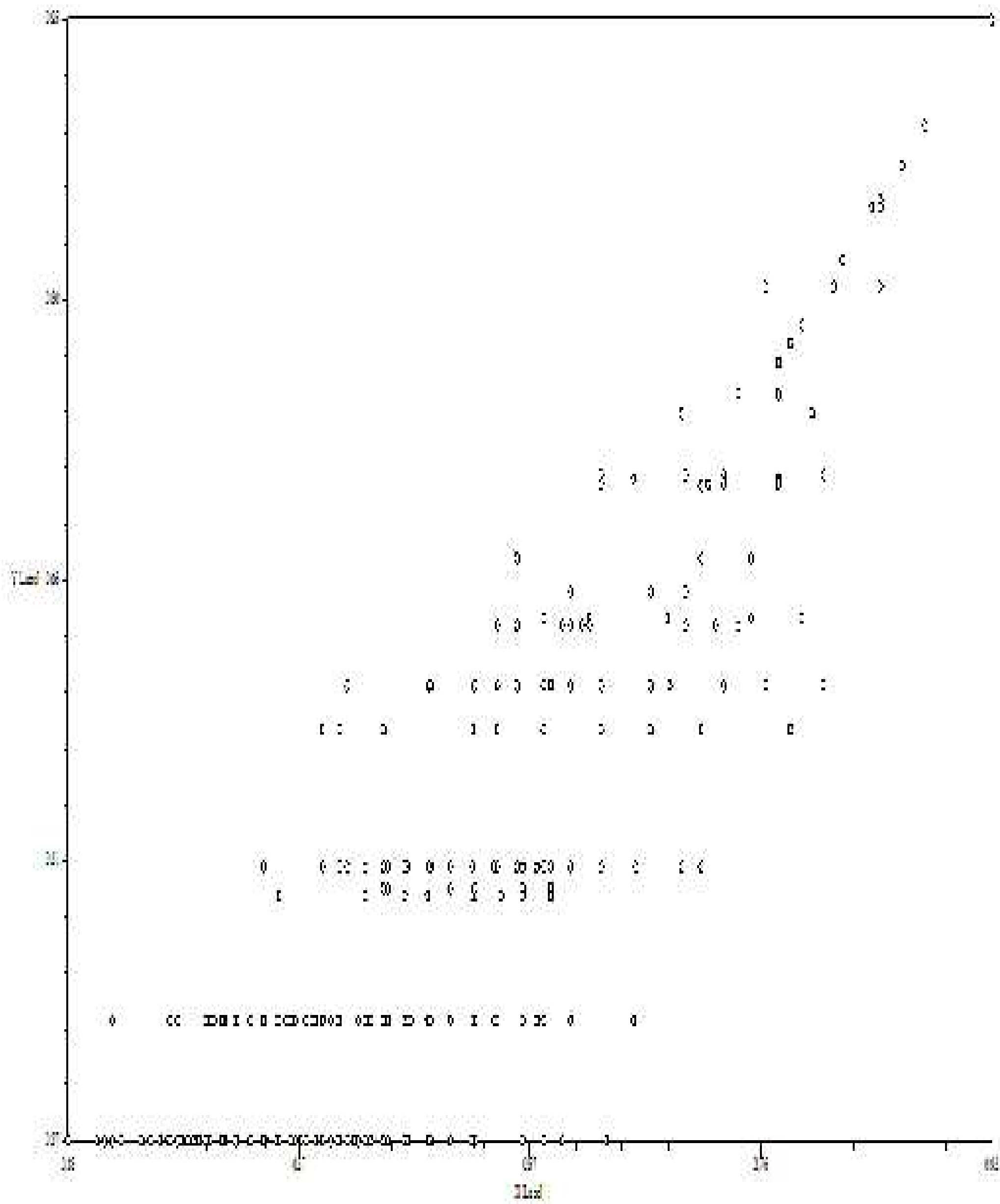


Fig 4.17: Scatter plot showing cophenetic correlation of SSR analysis

4.5.2.5 Cophenetic correlation study

Correlation study was carried out to compare the correlation of original similarity matrix of SSR results with the dendrogram clustering pattern. Using the COPH module of NTSYSpc version 2.02, r value was calculated and results were also expressed graphically (Fig 4.17). High correlation between the similarity matrix and dendrogram pattern was justified by the 'r' value which was found to be 0.87 which is good to fit.

4.5.3 Pooled Study of Molecular Markers:

Based on molecular marker study through RAPD and SSR, genotypes can be differentiated from one another to some extent. The reliability of one particular marker does not fulfill the goal of identification of varieties. So the pooled cluster analysis using three molecular markers to confirm the difference and similarity between genotypes obtained in earlier study.

4.5.3.1 Genetic similarity

Genetic similarity of two molecular markers were determined for each pair of thirty genotypes which revealed the lowest similarity of 0.39 was noticed between Red okra and Parbhani kranti, while highest of 0.88 was noticed between AOL 13-90 and AOL 12-59 (Table 4.15).

4.5.3.2 Cluster Analysis of RAPD and SSR

Jaccard's similarity coefficient and UPGMA method were used to develop a dendrogram (Table 4.15 and Figure 4.18) which divided into main two cluster-A and cluster-B.

In major cluster A comprised in A1 and A2 consisted of two groups A_{11} and A_{12}. Group A_{11} consisted of twelve genotypes *viz*., AOL 13-144, AOL 13-88, AOL 13-112, AOL 14-08, AOL 13-90, AOL 12-59, AOL 13-07, Kashi Kranti, Pusa Sawani ,AOL 12-52, AOL 13-73 and AOL 12-55, group A_{12} consisted of *viz*., AOL 14-32,AOL 13-94, AOL 09-02, AOL 14-29, JOL 11-12 and Parbhani Kranti, while subcluster A2 group A_{13} consisted of *viz*., GP OK-296, GP OK-292 and GP OK-213. Major cluster comprised in two groups, group B_{11} consisted of five genotypes *viz*., AOL 13-141, JDNO 11-11, AOL 14-11, JDNO 11-12 and AOL 13-133 ,while group B_{12} consisted of three genotypes GAO-5, JOL 13-05 and JOL 09-05. In cluster B_{12} separately comprised in only one genotype is Red okra that was most diverse among all the thirty genotypes.

To test the goodness of fit of the clustering of RAPD and SSR data, matrixes of cophenetic values were also computed using the program COPH (Fig 4.19). 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 = 0.862) as categorized by Rohlf (1998) was found under the category of **"good fit"**

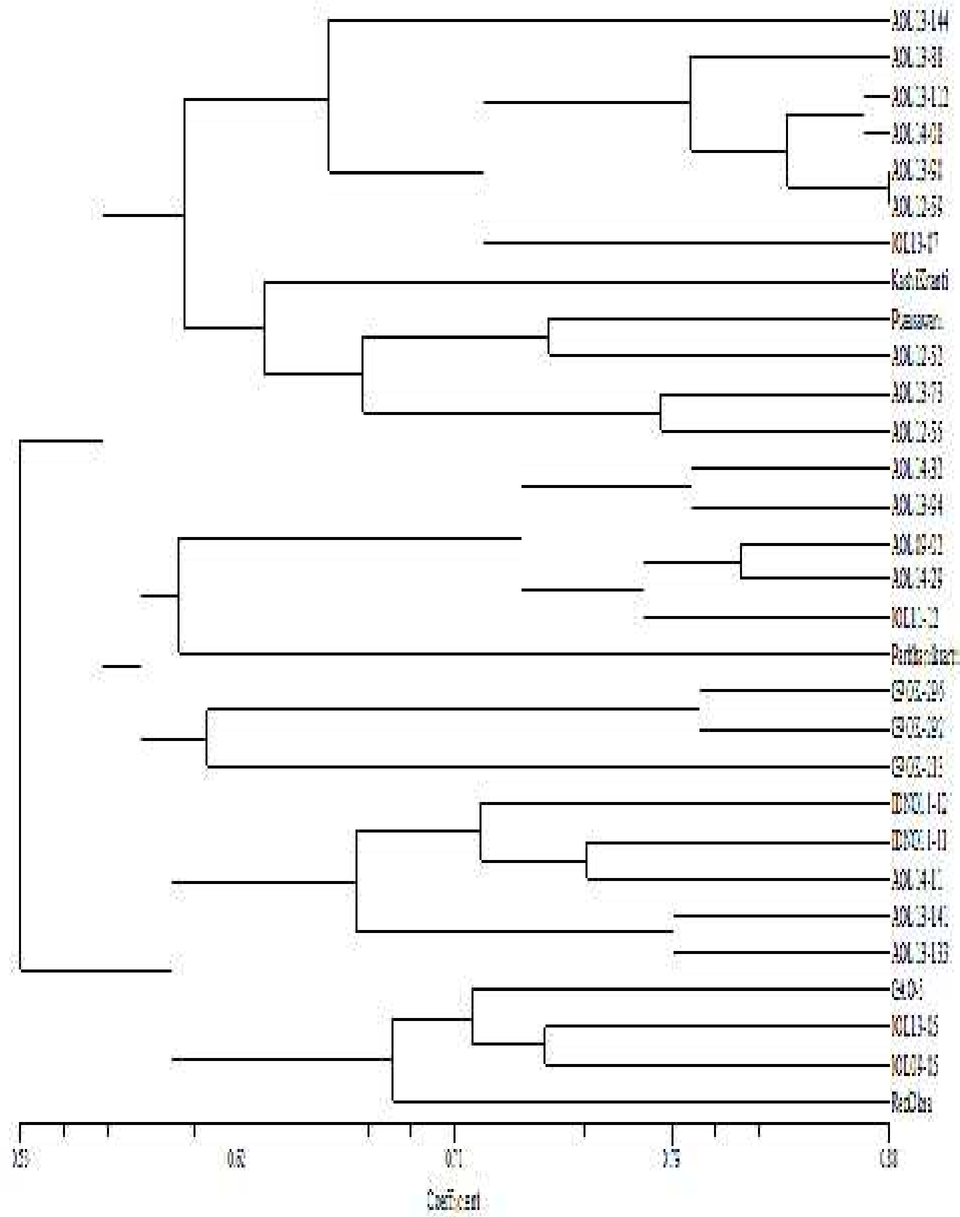


Fig. 4.18: Dendogram showing clustering of thirty okra genotypes constructed using UPGMA based on Jaccard's coefficient obtain from pooled data analysis

Table 4.15: Genetic similarity matrix of pooled data based on Jaccard's similarity coefficient

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1	1.00																													
2	0.61	1.00																												
3	0.64	0.60	1.00																											
4	0.69	0.66	0.72	1.00																										
5	0.65	0.67	0.60	0.62	1.00																									
6	0.61	0.70	0.75	0.60	0.71	1.00																								
7	0.57	0.66	0.59	0.69	0.67	0.73	1.00																							
8	0.55	0.62	0.65	0.65	0.64	0.71	0.72	1.00																						
9	0.53	0.46	0.51	0.56	0.56	0.55	0.71	0.76	1.00																					
10	0.57	0.62	0.55	0.55	0.60	0.54	0.66	0.65	0.72	1.00																				
11	0.56	0.45	0.47	0.51	0.54	0.49	0.57	0.66	0.74	0.79	1.00																			
12	0.47	0.59	0.58	0.61	0.62	0.58	0.57	0.62	0.58	0.60	0.61	1.00																		
13	0.58	0.55	0.54	0.49	0.46	0.52	0.53	0.62	0.58	0.65	0.75	0.54	1.00																	
14	0.58	0.50	0.47	0.45	0.42	0.49	0.48	0.59	0.56	0.60	0.70	0.49	0.74	1.00																
15	0.63	0.54	0.49	0.49	0.45	0.48	0.44	0.55	0.51	0.57	0.65	0.44	0.60	0.74	1.00															
16	0.52	0.63	0.57	0.57	0.63	0.57	0.52	0.55	0.47	0.57	0.51	0.60	0.53	0.48	0.61	1.00														
17	0.49	0.66	0.58	0.58	0.62	0.59	0.53	0.60	0.51	0.57	0.54	0.56	0.54	0.47	0.59	0.60	1.00													
18	0.52	0.54	0.54	0.56	0.62	0.53	0.49	0.52	0.45	0.51	0.46	0.52	0.48	0.59	0.51	0.64	0.56	1.00												
19	0.53	0.54	0.50	0.53	0.52	0.52	0.48	0.52	0.45	0.46	0.45	0.42	0.52	0.49	0.51	0.57	0.61	0.61	1.00											
20	0.58	0.66	0.61	0.61	0.57	0.54	0.50	0.52	0.45	0.50	0.47	0.51	0.47	0.49	0.52	0.58	0.65	0.65	0.70	1.00										
21	0.61	0.73	0.70	0.62	0.62	0.64	0.52	0.59	0.40	0.47	0.40	0.47	0.47	0.45	0.51	0.55	0.60	0.59	0.69	0.74	1.00									
22	0.52	0.60	0.57	0.55	0.62	0.62	0.56	0.62	0.52	0.59	0.55	0.55	0.49	0.44	0.48	0.59	0.60	0.61	0.58	0.74	0.69	1.00								
23	0.62	0.53	0.52	0.56	0.56	0.55	0.55	0.58	0.58	0.56	0.56	0.50	0.52	0.58	0.55	0.51	0.56	0.58	0.65	0.72	0.68	0.79	1.00							
24	0.63	0.60	0.59	0.61	0.60	0.62	0.58	0.53	0.49	0.52	0.51	0.55	0.49	0.52	0.54	0.54	0.57	0.57	0.55	0.64	0.59	0.68	0.71	1.00						
25	0.64	0.59	0.56	0.56	0.56	0.62	0.61	0.54	0.47	0.57	0.51	0.47	0.49	0.54	0.57	0.49	0.55	0.52	0.55	0.62	0.60	0.61	0.67	0.62	1.00					
26	0.64	0.62	0.56	0.56	0.57	0.60	0.60	0.54	0.50	0.60	0.52	0.50	0.49	0.54	0.60	0.56	0.54	0.51	0.54	0.59	0.58	0.62	0.66	0.68	0.67	1.00				
27	0.64	0.57	0.57	0.57	0.57	0.60	0.60	0.53	0.47	0.56	0.49	0.55	0.47	0.52	0.56	0.50	0.51	0.55	0.55	0.62	0.58	0.62	0.65	0.62	0.66	0.85	1.00			
28	0.68	0.62	0.61	0.56	0.57	0.62	0.64	0.54	0.50	0.60	0.52	0.52	0.54	0.57	0.60	0.51	0.54	0.51	0.54	0.62	0.60	0.57	0.66	0.76	0.65	0.85	0.88	1.00		
29	0.60	0.62	0.58	0.56	0.58	0.55	0.49	0.54	0.45	0.49	0.45	0.47	0.49	0.62	0.59	0.49	0.55	0.46	0.55	0.62	0.62	0.55	0.60	0.72	0.70	0.75	0.73	0.75	1.00	
30	0.59	0.52	0.47	0.55	0.48	0.55	0.55	0.68	0.42	0.64	0.66	0.51	0.66	0.66	0.72	0.55	0.56	0.50	0.52	0.59	0.62	0.59	0.65	0.64	0.65	0.65	0.66	0.65	0.67	1.00

(1:AOL 12-52, 2:AOL 14-32, 3:AOL 13-94, 4:AOL 09-02, 5:AOL 14-29, 6:JOL 11-12, 7:AOL 13-141, 8:JDNO 11-11, 9 Kashi Kranti, 10:JDNO 11-12, 11: AOL13-133, 12:JOL 09-06, 13:AOL 14-11, 14:JOL 13-03, 15:GP OK-296, 16:GP OK-213, 17:GP OK-291, 18: GAO-5, 19:Red Okra, 20:Pusa Sawani, 21:Parbhani Kranti, 22:AOL 13-73, 23:AOL 12-55, 24:AOL 13-38, 25: AOL 13-111, 26:AOL 14-06, 27 AOL 13-90, 28:AOL 12-59, 29:JOL 13-07 And 30 AOL 13-144)

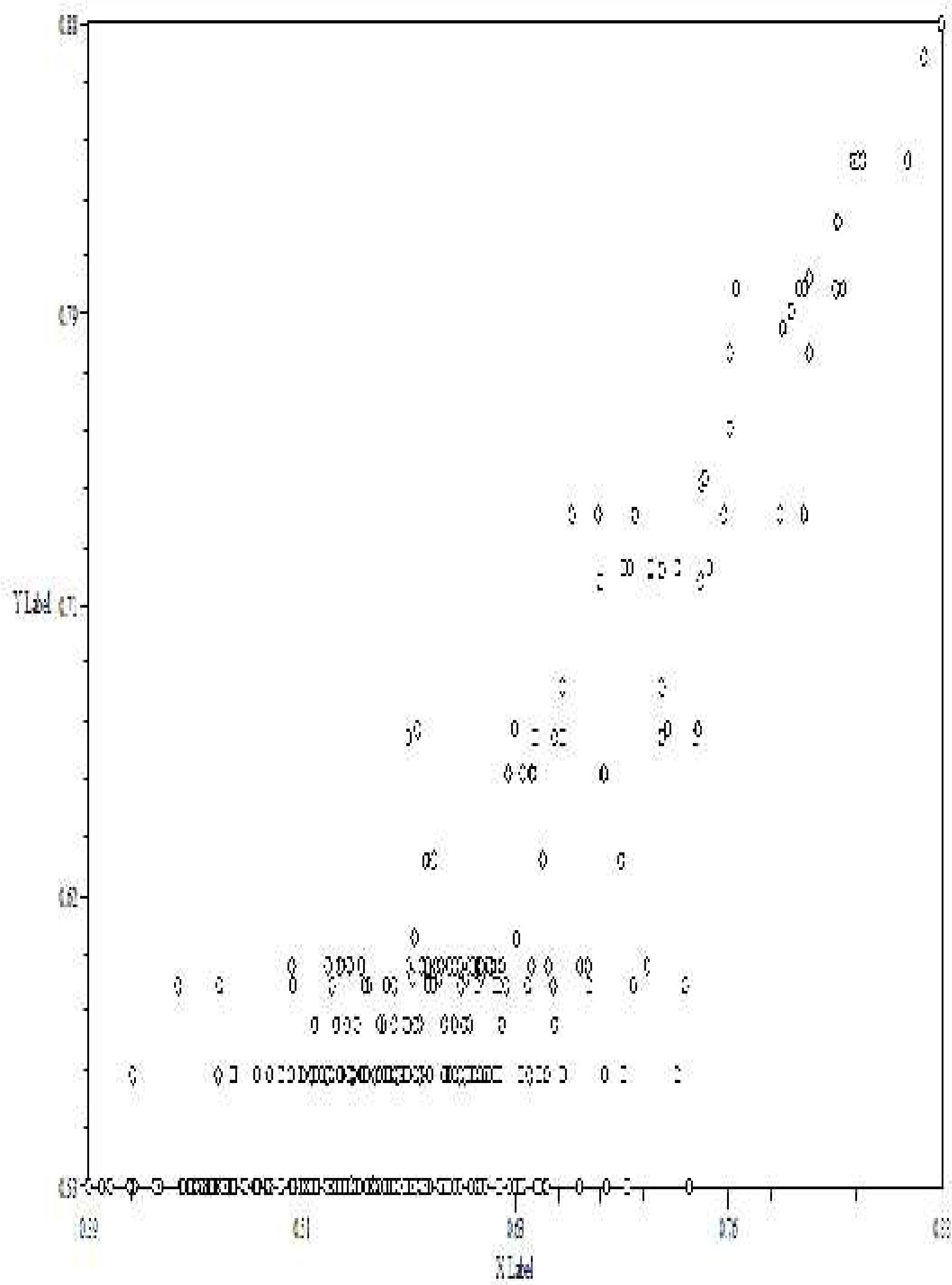


Fig. 4.19: Scatter-plot showing Cophenetic correlation of pooled data

5. SUMMARY AND CONCLUSION

The investigation on "Characterization of Okra (Abelmoschus esculentus L. Moench) Genotypes through Biochemical and Molecular Markers" was carried out at the Department of Biochemistry, BACA, AAU, Anand, Main Vegetable Research Station, AAU, Anand and Department of Animal biotechnology, Anand during 2015-2016.

Okra (*Abelmoscus esculentus* L.) is an important vegetable crop in India with highest production in the world. Gujarat produces 16 % okra production of total Indian production.

Morphological attributes like plant height, fruit length, fruit girth, and fruit weight fruit volume and fruit density was measured. Significantly the highest plant height was recorded for Red okra (127.06 cm) as compared to other okra genotypes. Fruit length was higher in Parbhani kranti (12.76 cm), which was followed by GP OK-292 (12.73 cm). Fruit girth (7.83 cm) and fruit weight (17.30 cm) was recorded higher in GP OK-292 as compared to other genotypes, while fruit volume and fruit density was higher in AOL 14-08 (10.46 cm^3) and (1.69 g/cm^3) respectively. This might be due to its genetic makeup and more adoptability towards environment.

Different biochemical attributes were measured from seeds of okra. The okra seeds have 2.38 – 7.02% moisture, 15.87 – 22.01% crude protein, 13.89- 18.93% true protein, 10.33 – 16.01% total lipids, 5.41 – 9.74% acid value, 2.91 – 4.90% free fatty acids.

Various phytochemicals *viz*., flavanoids, glycosides, terpenoids , tannins, alkaloids were qualitatively measured. The results shows that tannins and alkaloids are absent among all thirty genotypes, while flavanoids were present in all genotypes except JOL 11-12, JDNO11-11, JDNO11-12. Glycosides were present in all genotypes except AOL 14-29 ,GP OK-213, Pusa sawani, and the terpenoids were absent in AOL 12-52, AOL13-94, AOL 13-141, GP OK-213, GP OK-292 and parbhani kranti.

Variation in total protein banding pattern was obtained between thirty genotypes. Okra genotypes showed more number of fifteen numbers of bands. The genotypes Red okra were differentiated among all okra genotypes.

The isozyme of polyphenol oxidase showed amongst thirty genotypes and can be differentiated by bands, it represent as a intensity according to low, moderate and high, here in present investigation the high intensity of PPO isozyme present in AOL 13-133, moderate intensity in GP OK-296, Red okra, Parbhani kranti, AOL 12-55 and AOL 13-07 at 0.56 Rm value, while low intensity is present in all the remaining genotypes at 0.34 Rm value. In peroxidase the results showed total three bands of peroxidase isozyme with Rm value of 0.29,0.58 .Out of which, higher intensity of peroxidase is present in AOL 14-32,JOL 13-05, AOL 13-112 and AOL 13-144 ,moderate intensity present in AOL 12-52, AOL 09-02, AOL 14-29,JOL11-12,JDNO 11-11, GP OK-296, GAO-5,AOL 13-73,AOL 12-55,AOL 13-88,AOL 13-90 and JOL 13-07 while low intensity is present in rest of all genotypes at 0.58 Rm value. In 0.29 Rm value thirteen genotypes have absent bands of peroxidase, while in esterase isozyme the results showed

total three bands of Esterase isoenzyme with Rm value of 0.25, 0.27, 0.53, 0.56, 0.60 and 0.65 .In Rm value 0.60 and 0.65 were Pusa sawani and Parbhani kranti are absent respectively, while single band is present in all the genotypes at 0.25, 0.27, 0.53 and 0.56 Rm value.

The RAPD analysis with 8 primers produced 72 total loci and total bands produced were 1074. Out of the 72 loci produced, 55 were polymorphic and hence the total polymorphism percentage was found to be 74.60%. Average number of loci per primer was found to be 9.0 average number of polymorphic loci obtained per primer was found to be 6.87. The molecular size of the amplified PCR products ranged from 185bp (OPK-08) to 1926bp (UBC-465). The highest similarity index value 0.91 was found between AOL 13-90 and AOL 12-59, while the least similarity index value 0.45 was found between JOL09-05 and Red okra. The average similarity coefficient among genotypes was 0.730. The minimum (87) and maximum (220) number of bands were produced by UBC-465 and OPC-8 respectively. The highest polymorphism (100%) was produced by UBC-465.The highest PIC value obtained was 0.880 for OPC-8 and lowest PIC value obtained was 0.756 for UBC-509. Marker Index value for polled RAPD data was found to be 4.07.

The Clustering pattern of thirty okra genotypes was generated based on UPGMA-dendogram using the pooled molecular data of eight RAPD. The results indicated that two clusters namely A and B were formed at a similarity coefficient of 0.59. Cluster A was divided into two sub-cluster A_1 and A_2. A_1 cluster further separate into A_{1a} and A_{1b} which is consisted of AOL 13-144,AOL

14-32,AOL13-94, AOL09-02, JOL11-12, JDNO 11-11, AOL14-29, AOL 13-141, Kashi kranti, GAO-5,Pusa Sawani, AOL 12-52,AOL 13-73, AOL 12-55, AOL 13-88, AOL 13-112, AOL 13-90, AOL 12-59, AOL 14-08, JOL 13-07, JOL 09-05, while A2 sub-cluster consisted of JDNO 11-12,AOL 13-133,Parbhani Kranti, AOL 14-11, JOL 13-05, GP OK-296, GP OK-213 ,GP OK -292.Major cluster B consisted of Red okra. Correlation study was carried out to compare the correlation of original similarity matrix of RAPD results with the dendrogram clustering pattern. High correlation between the similarity matrix and dendrogram pattern was justified by the 'r' value which was found to be 0.831 which is good to fit.

In the SSR analysis 15 markers were amplified and produced 71 alleles. The average number of alleles per locus was found to be 4.73, while effective number of alleles was 1.46. The maximum number of alleles was 8 which were recorded for markers OK-8, whereas OK-1, OK-6, OK-11, OK-12 and OK-14 were produced three alleles. The highest allele frequency recorded in OK-6 (0.66) primer. The highest PIC value was recorded for OK-7 (0.42) and lowest value was recorded for OK-1, OK-3, OK-11 and OK-15 (0.67). The average PIC value and number of alleles were 0.514 and 4.8, respectively. The molecular weight of the amplified PCR products ranged from 101 (OK-2) to 226 (OK-1). The highest similarity index value of 0.95 was found between AOL 13-141 and AOL 13-133 genotypes, while the least similarity index value of 0.15 was found between JOL 13-05 and AOL 14-29. Marker Index value for pooled SSR data was found to be 5.78.

Clustering pattern of dendogram was generated by using the pooled molecular data of 15 SSR loci indicated that two clusters were formed at a similarity coefficient of 0.54. Clustering pattern of dendrogram generated by using the pooled molecular data of 15 SSR loci indicated that two clusters namely A and B Cluster A was divided into two sub clusters A_1 and A_2. Grouping of twenty two genotypes that are in one major cluster 'A'. Sub-cluster A1 included okra genotypes *viz*., AOL 12-52,AOL 14-32, AOL 13-94,AOL-09-02, AOL 14-29, JOL 11-12, Kashi Kranti, GP OK-292, GP-OK-213, GP OK-296, Red okra, GAO-5, Pusa Sawani, AOL 13-73 and AOL 12-55. Sub-cluster A2 consists of genotypes AOL 13-112, AOL 14-08, AOL 13-90, AOL 12-59, JOL 13-07, AOL 13-144 and AOL 13-88. Cluster B was divided into two sub-clusters B1 and B2. Grouping of eight genotypes that are one major cluster 'B'. Sub-cluster B1 consists of genotypes *viz*; Parbhani Kranti, AOL 13-141, AOL 13-133, JDNO 11-11, AOL 14-11 and JDNO 11-12; whereas sub-cluster B2 consists of genotypes *viz*., JOL 13-05 and JOL09-05. Correlation study was carried out to compare the correlation of original similarity matrix of SSR results with the dendrogram clustering pattern. High correlation between the similarity matrix and dendrogram pattern was justified by the 'r' value which was found to be 0.870 which is good to fit.

According to RAPD and SSR study in RAPD marker total number of eight primers are studied among that only few of primers *viz*.OPC-8, OPC-19, OPY-4, OPAE-15, UBC-465 are differentiate the Red okra genotype among thirty genotypes according to dendrogram clustering pattern of RAPD , while

in SSR marker total fifteen primers were studied ,only OK-7 and OK-15 primers are clearly distinguish the red okra is diverse than others ,so it can be justified as Red okra is most diverse genotype among thirty genotypes.

Genetic similarity of two molecular markers (RAPD & SSR) was determined for each pair of thirty genotypes. The present study indicated a fairly high level of polymorphism with similarity coefficient ranging from 0.39 to 0.88 indicating a fairly wide and diverse genetic base.

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