"MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF OKRA CULTIVARS *(Abelmoschus esculentus* L.*)*"

A

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"MORPHOGOLICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF OKRA CULTIVARS (*Abelmoscus esculentus* L.)"

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ABSTRACT

Okra (*Abelmoschus esculentus* L.) (2n=130) is known in many english-speaking countries as lady's fingers or gumbo, and it 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.

Growth and yield attributes like plant height, fruit length, fruit girth, fruit volume, fruit density and fruit yield were measured. The population of fruit and shoot borer and fruit borer were recorded among different okra cultivars at 35 and 60 days after sowing. The results can help to differentiate susceptible and resistant cultivars.

Different biochemical components like moisture, total carbohydrates, mucilage, crude fiber, total soluble sugars, reducing sugars, non reducing sugars, total phenols, true protein, total antioxidant activity, ascorbic acid, chlorophyll a,

chlorophyll b, chlorophyll a/b, total chlorophyll and total carotenoids were measured from fresh fruits of okra.

The activity of peroxidase, polyphenol oxidase and catalase were measured. The activity for three enzymes was recorded highest in cv. GAO-5 and lowest in cv. Glory. Isozyme electrophoresis of peroxidase, superoxide dismutase and polyphenol oxidase were found useful for identification of cultivars as well as may serve as marker isozyme for okra cultivars. In peroxidase maximum number of bands were found in GAO-5 (6) while in polyphenol oxidase maximum number of bands were observed in Arka Anamika, Nirmala-303 and GAO-5 (3). In SOD maximum number of bands were found in Ankur-40 and Arka Anamika (6) while minimum number of bands were found in GAO-5(3).

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) of fruits proteins showed variability, and could be effectively used for genetic diversity of okra cultivars on the basis of variations in banding pattern and intensity of bands.

The molecular characterization of okra cultivars was done by using polymerase chain reaction (PCR) based molecular markers, and simple sequence repeat (SSR). In the SSR analysis 8 markers were amplified and produced 19 alleles. The average number of alleles per locus was found to be 1.87, while effective number of alleles was 2.37. The maximum number of alleles was seven which were recorded for marker OK – 2.

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CERTIFICATE

This is to certify that the thesis entitled **"MORPHOLOGICAL, BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF OKRA CULTIVARS (*Abelmoschus esculentus* L.)"** submitted by **JAPDA ASHISHKUMAR RANMALBHAI** in partial fulfillment of the requirements for the award of the degree of **MASTER OF SCIENCE** in **BIOCHEMISTRY** of the Anand Agricultural University is a record of bonafide research work carried out by him 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: /07/2014 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 **Biochemistry**, by the undersigned is the results of investigation done by him 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
Date: /07/2014 (Japda Ashish R.)

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Place: Anand
Date: /07/ 2014 *(Japda Ashish R.)*

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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) h	Hours
21)ha	Hectare
22)HCl	Hydrogen Chloride
23)hrs	Hours
24) HSP	Heat shock protein
25) ISSR	Interspersed simple sequence repeat

26)Kb	Kilo base
27) Kg	Kilo gram
28)M	Molar
29)mA	MilliAmpere
30)mg	Milligram
31)MgCl	Magnesium Chloride
32)min	Minutes
33) ml	Mili litre
34) Mm	Mili meter
35)mM	Milli Molar
36)mol wt	Molecular weight
37)N	Normal
38)NaCl	Sodium Chloride
39)ng	Nanogram
40) ng/l	Nanogram per litre
41) nm	Nano meter
42)NMR	Nuclear Magnetic Resonance
43)No.	Number
44)OD	Optical Density
45)PCR	Polymerase Chain Reaction
46) Pg	Picogram
47)pH	Negative logarithm of Hydrogen ion concentration
48)PIC	Polymorphism Information Content
49) POX	Peroxidase
50) PPO	Polyphenol oxidase
51) PR	Pathogenesis related proteins

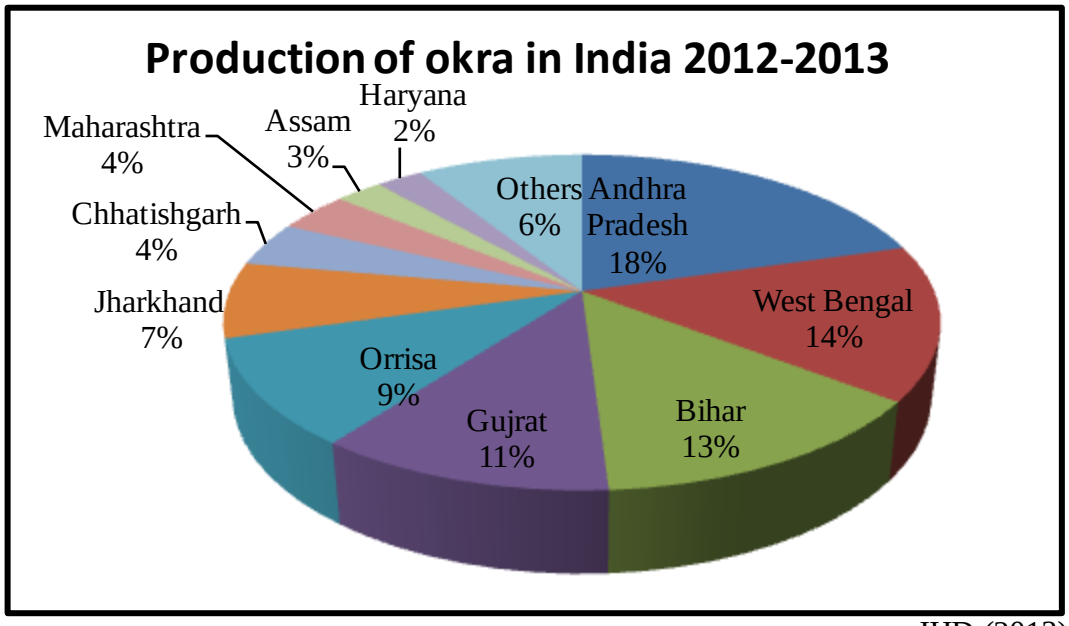
52) PVP	Polyvinylpyrolidone
53)RAPD	Randomly Amplified Polymorphic DNA
54)SAHN	Sequential Agglomerative Nested Cluster
55) SBS	Sequencing-by-synthesis
56) SEm	Standard error of mean
57)SIMQUAL	Similarity for Qualitative data
58) SNP	Single nucleotide polymorphism
59) SOD	Superoxide dismutase
60)Sr.	Serial
61) SSR	Simple sequence repeat
62) T	Temperature
63) t	Ton
64)TBE	Tris-Borate EDTA
65)TE	Tris- EDTA
66)U	Units of enzyme
67)UPGMA	Unweighted Paired Group Mathematical
68)viz	Namely
69)w/v	Weight per Volume
70)µg	Microgram

CHAPTER – I

INTRODUCTION

Okra (*Abelmoschus esculentus* L.) (2n=130) is known in many countries as lady's fingers or gumbo and it is a flowering plant in the *Malvaceae* family. It is valued for its edible green pods. Originated in Africa, the crop is extensively cultivated in tropical, subtropical and warm temperate regions around the world. It is grown commercially in India; it is a popular vegetable crop and widely accepted due to its high nutritional value and pleasant flavour (Lamont Jr., 1999).

There are significant variations (2n = 26 to 130) in the chromosome numbers of okra and at ploidy levels of different species in the genus *Abelmoschus*. The lowest number reported is 2n=56 for *Abelmoschus angulosus* (Ford, 1938), whereas the highest chromosome number reported are close to 200 for *Abelmoschus manihot* var. caillei (Singh and Bhatnagar, 1975; Siemonsma, 1982a, 1982b).



IHD (2013)

Okra is mainly muliplied 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 axillary 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.

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 pods 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, nictonic 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).

Okra mucilage refers to the thick and slimy substance found in fresh as well as dried pods. Mucilaginous substances are usually concentrated in the pod walls but not in seeds and it is chemically acidic polysaccharide associated with proteins and minerals (Woolfe *et al.*, 1977). Although nature of the polysaccharides varies greatly,

neutral sugars such as rhamnose, galactose and galacturonic acid have been reported by many scientist (Hirose *et al.*, 2004; Sengkhamparn *et al.*, 2009).

Okra mucilage has potential for use as food, non-food products, and medicine. Mucilages and gums are water soluble polysaccharides found in a widespread number of plants and also in some microorganisms. It is popular in Indian and Pakistani cuisine, where chopped pieces are stir-fried with spices, pickled, salted or added to gravy-based preparations such as bhindi ghosht and sambar. In India, it is also used in curries. Woolfe and his colleagues (1977) have reported that the okra contains 1.6 g mucilage/100gm of okra. The sugars identified from 100 gm mucilage were rhamnose (0.12 %), galactose (1.00 %), glucose (0.10 %) and uronic acid identified as galactouronic acid (1.30 %)

Due to the rapid developments in the field of molecular genetics, varieties of different techniques have emerged to analyze genetic variation during the last few decayed.

The use of various DNA based molecular marker tools like Randomly Amplified Polymorphic DNA (RAPD), Inter Simple Sequence Repeats (ISSR), Simple Sequence Repeats (SSR) and Sequence Related Amplified Polymorphism (SRAP) are receiving much attention than morphological characterization for the evaluation of genetic diversity. They have large number of applications like characterization of gene pool, DNA fingerprinting, phylogenetic analysis, molecular dissection of complex traits, and characterization of genome organization.

The various biochemical methods like protein profiling and isoenzymes are useful for varietal identification. The DNA based molecular marker methods such as RAPD, ISSR and SSR are rapid, relatively cheap, eliminate the need of grow out test.

Because of high mucilage content of okra it is very difficult to extract protein and nucleic acid. Very little work has been done in this crop. Keeping in view the above facts the present experiment is planned to study the genetic diversity among genotypes of okra with following objectives.

Objectives:

1) To study morphological and biochemical attributes of okra cultivars for its nutraceutical components
2) To identify biochemical and molecular markers for okra cultivars
3) To study associationship between morphological and biochemical components

CHAPTER II

REVIEW OF LITERATURE

The current literature represents summary of research work related to physiological, biochemical and molecular markers among okra cutivars. 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:

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.

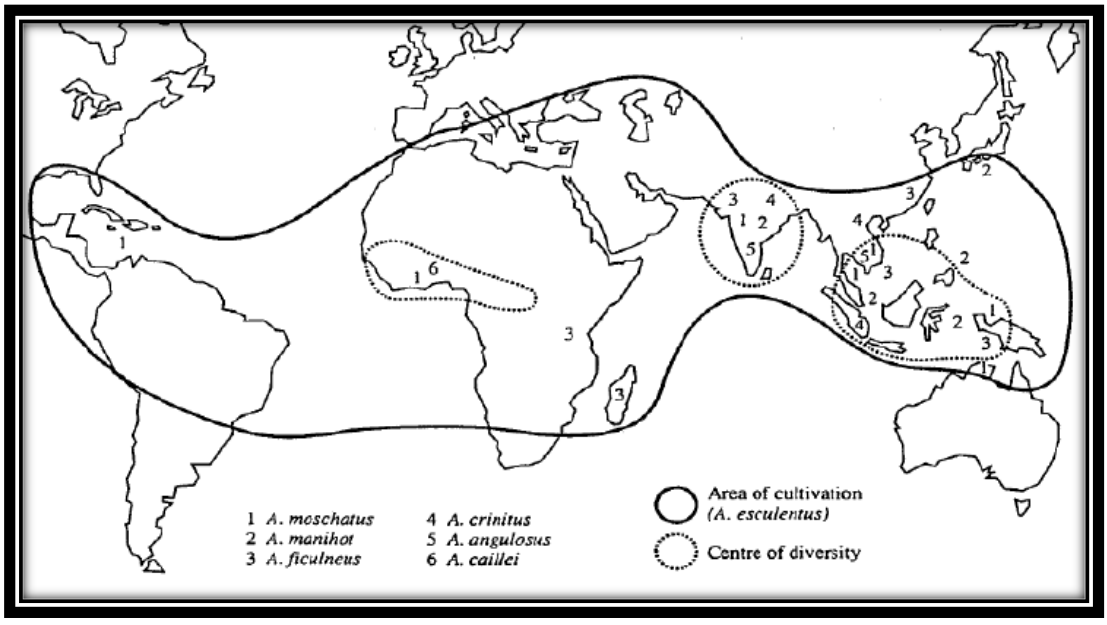


Fig 2.1 Geographical distribution of cultivated and wild species.

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. *A. 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. criniturs*	Uttaranchal, Madhya Pradesh, Orissa
4	*A. ficulneus*	Jammu & Kashmir, Rajasthan, Madhya Pradesh, Chhattisgarh, Maharashtra, Tamil Nadu, Andhra Pradesh, Uttar Pradesh
5	*A. manihot*ssp. tetraphyllus var. *tetraphyllus*	Uttar Pradesh, Rajasthan, Madhya Pradesh, Maharashtra, Orissa, Chhattisgarh
6	*A. manihot ssp. 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 GROWTH and YIELD 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).

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).

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. The plant height of okra ranged between 38 -147.65 cm in 161 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).

Dhruve *et al.* (2011) examined the bioefficacy of silixol (stabilized form of silicic acid) on okra. They concluded that the plant height was improved by foliar application of silixol as compared to control.

2.2.2 Fruit length (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.

Salimath *et al.* (2011) observed that the fruit length of okra ranged between 9.1 – 17.35 cm in 161 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.

Kabir and Pillu (2011) observed that the okra fruit length was maximum 11.83cm in Shagun followed by 11.75cm in Arka Anamika and 11.16cm in Sinnova. The fruit length of okra was ranged between 9 to 23 cm. (Kasrawi *et al.*, 2007).

Talib *et al.* (2012) studied role of different physico-chemical characters of nine cultivars of okra plant among those, the highest fruit length was observed with Diksha (12.70 cm), while the lowest fruit length was with Pusasawani (8.90 cm).

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 highest 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) 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 highest 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).

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 *et al.* (2012) studied the performance of different okra (*Abelmoschus esculentus* L.) cultivars under the agro-climatic condition of Dera Ismail Khan and they recorded the fruit weight was ranged between 8.23 gm (Puja) to 10.33 gm (Sabzpari).

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.

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_2 NPK 30, 20, 20)13.99 gm during the study on effect of fertilizer on growth parameters of okra plant.

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.

2.2.6 Fruit density (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.

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

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

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.

2.2.7 Fruit yield (q/ha)

Tapankumar and Pradyumna (2009) studied performance of okra hybrids under reduced level of chemical fertilizers (50% of recommended dose of fertilizers) supplemented with organic manures. They reported the maximum and the minimum fruit yield with varieties NOH 15 (107.98 q/ha) and Panchali (74.30 q/ha), respectively.

Kamrun *et al.* (2009) reported that different sowing times had significant effect on the fruit yield per hectare and in their study the highest fruit yield per hectare (13.88 t) was obtained from April sowing, while the lowest fruit yield per hectare

(10.22 t) was observed from March sowing, which was 30.07% lower than April sowing.

Weerasinghe *et al.* (2010) observed that the total yield (kg/ha) was significantly affected by fertilizer treatments. The highest total fruit yield per hectare was recorded with T_1 (DOA recommendation/plant) 8.5 t/ha, while the lowest fruit yield per hectare was obtained from treatment T_2 (DOA recommendation/unit) 6 t/ha.

Attigah *et al.,* (2013) studied the treatments effect on yield parameter of okra in which higher fruit yield observed in T_4 (½ N P K + ½ Cow dung manure) 4527 kg/ha.

Mishra *et al.* (2013) recorded the maximum fruit yield (228.38 q ha-1) in T_6, (FYM@10 t/ha-1 100% RDF + vermicompost @5 t/ha + biofertilizer) which was significantly superior to rest of the treatments tried in the experiment. However, the lowest yield (80.24 q ha-1) was recorded in T_1 (Control + FYM@10 t/ha-1).

Basar *et al.* (2011) recorded the highest fruit yield in S_4F_2 (S_4 spacing 20cm ; F_2 NPK 60,40,40) treatment (15.76 t/ha) during the study on effect of fertilizer on growth parameters of okra plant.

2.3 PEST DAMAGE STATUS:

Okra crop is attacked by several insects pests; of which eleven species of insects have been recorded in Gujarat (Patel *et al.*, 1970). Amongst these, the major insect and non insect pests are fruit borer; shoot and fruit borer, *Earias vittella* (Fabricius); Jassid, *Amrasca biguttula biguttula* (Ishida); whitefly, *Bemisiatabaci* (Gennadius); aphid, *Aphis gossypii* (Glover) and spider mite, *Tetranychus cinnabarinus* (Boisduval), these insect pests infesting okra crop during different growth stages and seasons.

The losses caused by insect pests in okra was estimated by various workers. Ghosh *et al.* (1999) reported 30.81 per cent avoidable losses in fruit yield caused by insect pests at Coochbehar, West Bengal. Similarly, Kanwar and Ameta (2007) had also reported about 49.97 % losses in fruit yield due to insect pests of okra with 8.92, 15.69 and 49.30 percent reduction in plant height, number of effective branches per plant and marketable fruit yield, respectively.

2.3.1 Fruit and shoot borer

Vyas and Patel (1991) reported that shoot damage due to *E. vittella*in okra was lower in selection-2 (3.00%), which was statistically at par with Parbhani kranti (4.80%), Padra 18-6 (5.45%) and Gujarat Okra-1 (6.20%). While fruit damage caused by *E. vittella* was lower in Gujarat Okra-1 (21.90%), which was statistically at par with Selection-2 (22.50%), Parbhanikranti (23.34%) and Punjabpadmini (23.45%). On the basis of damage caused by *E. vittella* to fruits and the marketable fruit yield, Gujarat Okra-1 was considered as the most suitable for use under the prevailing climatic conditions of Gujarat.

Mandal *et al.* (2006) screened the relative susceptibility of 7 okra cultivars *viz,* ArkaAbhay, ArkaAnamika, D-1-87-5, D-1-87-16, HRB-55, KS-312 and PusaSawani against *E. vittella* under field conditions, and reported the minimum shoot infestation (8.7%) with variety D-1-87-5, which was statistically at par with ArkaAnamika (9.4%), whereas, the maximum shoot infestation (11.9%) was observed in Pusa Sawani.

Patel *et al.* (2012) screened 10 different cultivars of okra for their susceptibility to *E. vittella* under field condition, in which they found the lowest shoot borer infestation in AOL-05-1 (3.03%) and the highest shoot borer infestation in AOL-08-10 (11.83%).

2.3.2 Fruit borer

Neeraja *at el.* (2004) reported that the incidence of fruit borer in two varieties of okra was 21.7% (MBORH-913) and 29.2% (Vijaya).

The percent damage in fruit was studied by Naresh *et al.* (2003). They observed the lowest fruit damage in Hybrid no. 8 followed by Jaya, Okra Hybrid-1, Arkaabhay, Harsha, Vijaya, Arkaanamika and Saumya. Based on shoot damage, Vijaya was comparatively less susceptible than the other cultivars.

Mandal *et al.* (2006) screened relative susceptibility of 7 okra cultivars *viz,* ArkaAbhay, ArkaAnamika, D-1-87-5, D-1-87-16, HRB-55, KS-312 and PusaSawani against *E. vittella* and found that PusaSawani was the most susceptible with 37.2% fruit infestation.

Patel *et al.* (2012) screened 10 different cultivars of okra for their susceptibility to *E. vittella* under field condition, in which they found the lowest fruit borer infestation in AOL-05-1 (7.50%) and the highest fruit borer infestation in AOL-08-10 (22.70%).

2.4 BIOCHEMICAL ATTRIBUTES:

2.4.1 Moisture

Kabir and pillu (2011) observed that the moisture content increased up to 6^{th} day of fruit set, then it marginally decreased. The average moisture of cultivar Sinnova (90.56%) and Arka Anamika (90.54%) was significantly (0.05%) higher than Shagun(89.97%).

Fabunmi and Babarinde (2009) reported that the okra fruit contained 88% moisture, after nine days of storage, the moisture content of okra ranged between 84.4% and 85.4% for stored samples under refrigerating condition. For stored samples in the room temperature, moisture content ranged between 83.5% and 87.8%.

Rusea *et al,* (2014) showed that the percentage of moisture content of young fruit was 88.47%, it was the highest moisture content, while mature fruit had the lowest (82.25%) and leaves had 82.60% moisture content in okra.

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 observed that average seed maturity, Higairat had the highest moisture content (53.6%). On the other hand, Afreeta had the lowest moisture content (29.88%).

2.4.2 Total carbohydrates

Mannem *et al.* (2012) did quantitative evaluation of carbohydrate levels in green leafy vegetables. They found that curry leaves contained the maximum carbohydrate (0.935%) and spinach and lettuce contained the minimum carbohydrate (0.075%).

Rusea *et al.* (2014) observed that the carbohydrate content was the highest in the mature fruit (11.17%) followed by leaf at (8.83%) and young fruit (7.05%) in okra.

Nair *et al.* (2013) reported that okra fruits play a vital role to maintain sufficient carbohydrate level in the diet and also function as energy storage molecules. The total carbohydrate showed an increase upto 13 DAS in Arka Anamika, but tend to decrease later. They also observed that the okra fruit contain 3.49 mg/gm total carbohydrate in Arka Anmika cultivar.

Merina and Mary (2012) studied the effect of gibberellic acid on the counteracting of the Nacl 0, 50, 100 and 150 mm, which cause deleterious effects on okra leaves, in their study higher carbohydrates content (2.7%) was observed in 0 mm concentration of Nacl with gibbrellic acid.

Akenovat *et al.* (1982) studied changes in the carbohydrate constituents of okra (*Abelmoschus esculentus* L.) with age, and he recorded the variation from 11.2 to 13.1 g/100 g.

Azam khan *et al.* (2013) recorded the highest carbohydrates content in Pusa Selection (9.87 %) and the lowest in IN-97 (7.67 %) in okra during the study on genetic quality and nutrient uptake in okra under irrigated and wastewater ecosystem.

2.4.3 Mucilage

Woolfe *et al.* (1977) observed that okra fruit contained 16 gm mucilage/kg, while baobab leaves contained 18 gm mucilage/kg.

Nair *et al.* (2013) reported that the mucilage content was ranged between 0.147 to 0.363 mg/g in okra genotype Arka Anamika at different stages of fruit development.

Sharma *et al.* (2013) recorded the mucilage content of okra as 100mg/100gm.

Pathak and Parbhat (2012) studied genetic diversity and its relationship with heterosis in okra in which they reported 0.65% the mucilage content in okra.

Kumar *et al.* (2002) studied the performance of nutrient sources and its levels on hybrid bhendi under drip fertigation system and they recorded higher mucilage in T_2 (100 % RDF + N P K) 1.85 %.

2.4.4 Fiber content

Kabir and Pillu (2011) reported the crude fiber content of okra fruits increased significantly with increase in maturity irrespective of the cultivars. Crude fibre mean of Arka Anamika was the highest (4.88%), followed by Shagun (4.37) and Sinnova (3.64%).

Patel *et al.* (2012) studied the 10 different cultivars of okra in which higher fiber content found in AOL-05-1 (2.64%) and lower fiber content found in Parbhanikranti (0.49%).

Rusea *et al.* (2014) studied the fiber content in okra fruits and leaves, percentage of mean variation was observed as mature fruit 2.44%, young fruit 0.37% and leaves 1.13%.

Nair *et al.* (2013) found that okra genotype Arka Anmika contained 1.18 mg/gm fiber.

Akenovat *et al.* (1982) studied changes in the crude fiber constituents of okra (*Abelmoschus esculentus* L.) with age, and they reported a range of 12.5–16.7 g/100 g, and it was increased with age.

2.4.5 Total soluble sugars

Dhruve and his colleagues (2011) reported that foliar application of silicic acid in the form of silixol in okra improve the total soluble sugars as compared to the control.

Agbo (2014) studied the microbiological and nutritional quality of dried okra, and observed the maximum total soluble sugar in variety Yelen (1.34 mg/gm), while the minimum total soluble sugar sugar was found in Tomi (1.02 mg/g).

Rao and Sharma (2013) studied the metabolites of okra after infection of pest, they found that the total sugar was higher in pest infected (5.98 mg/gm) crop, while it was lower in pest free (5.38 mg/gm) crop.

Ruby *et al.* (1993) recorded total soluble sugar ranging between 2.28 – 5.22 g/100gm in okra fruit.

Merina and Mary (2012) studied the effect of the Nacl in different concentration (50, 100 and 150 mM), which induced deleterious effects on okra

leaves; they observed higher total soluble sugar content (9.87 %) in 150 mM concentration of Nacl, while lower soluble sugar content was in control (7.79 %).

2.4.6 Reducing sugars content

Talib *et al.* (2012) studied role of different physico-chemical characters of nine cultivars of okra among those the highest reducing sugar was observed in Ikra-1 (4.21%), while the lowest reducing sugar was in Sabzpari (2.72 %).

Ruby *et al.* (1993) recorded the reducing sugars ranging between 2.27-5.18 gm/100 gm in okra fruit.

Rahman *et al.* (2011) studied the effect of fertilizer on okra with different treatments in which they found that the highest reducing sugar content was with T_2 treatment (N_0P_1K1, 2 kg cow dung, 65 gm urea, 150 gm TSP, and 105 gm PM) (1.11 %).

2.4.7 Total phenol

Kabir and Pillu (2011) reported that phenol content in okra fruit of Arka Anamika (167.62mg/100g) was significantly higher than Sinnova (112.27mg/100g) and Shagun (106.26mg/100g).

Rusea *et al.* (2014) studied the status of total phenols in young leaves, mature leaves, young fruit and mature fruit. Okra leaves contained 0.99mg/0.1g phenols and the lowest was recorded with mature fruit as 0.13mg/0.1g in okra.

Nair *et al.* (2013) observed that the phenol content in okra cultivar Arka Anamika was ranged between 0.024 to 1.905 mg/gm.

Ali*et al.* (2009) recorded the total phenol content of leaves of okra as 68.81 mg/gm.

2.4.8 True protein

Hosagoudar and Chattannavar (2009) reported that cotton genotypes had differential reaction of grey mildew (*Ramularia areola* Atk.). They observed that at 90 days healthy plant of RCH-2 Bt contained the highest protein (6.839 mg/g), while JKCH-2 Bt contained the lowest protein (4.795 mg/g).

Rusea *et al.* (2014) studied protein content in 100g of okra fruits and leaves, results showed percentage of mean variation as with mature fruit (2.51%), young fruit contained (2.57%) and leaves (4.81%).

Nair *et al.* (2013) observed that okra fruit of Arka Anamika contained 0.948 mg/gm protein.

Arlai *et al.* (2009) observed that the conventional okra contained 5.2 % protein while the organic okra contained 11.5 % 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 g/100 g, and it was decreased with age.

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

2.4.9 Total antioxidant activity

Khomsug *et al.* (2010) observed that the pulp of okra had high relative antioxidant activity about 44.1mg/ml, and they also observed that phenolic compound were widely distributed in plant, which had gained much attention due to their antioxidant activities, which had beneficial effect on human health.

Ali *et al.* (2009) showed that the okra leaves (*Hibiscus esculentus*) contain 245 µg/ml antioxidant activity by the DPPH assay.

Yang *et al.* (2006) measured high levels of antioxidant activity (1024 µmol/100g FW) in okra relative to other common vegetables like carrot spinach and *aspargelous*, they used two different extractants (methanol and water) for their study.

2.4.10 Ascorbic acid content

Kabir and Pillu (2011) studied that ascorbic acid content of the different cultivars, it initially increased with maturity but later on with advancement of maturity it reduced significantly. Ascorbic acid content was found to be the maximum in Shagun (12.31mg/100g) followed by Arka Anamika (10.82mg/100g) and Sinnova (10.18mg/100g).

Fabunmi and Babarinde (2009) showed the effect of storage and packaging on ascorbic acid level of okra. The initial ascorbic acid content of fresh okra was 43.5 mg/100 g, however, ascorbic acid of all the samples decreased during storage.

Nair *et al.* (2013) studied the ascorbic acid content, it was increased steadily in the developing fruits of okra, which continued until fruit maturity and dehiscence suggesting that okra could be used as a very good source of ascorbic acid, ascorbic acid was found 0.922 mg/gm in Arka Anamika cultivar.

Arlai *et al.* (2009) studied the rate of vitamin C loss from the organic okra, and it was less than conventional okra, i.e., 14.1 and 58.1 (% wet basis), respectively.

2.4.11 Total chlorophyll

Hiremath *et al.* (2013) observed that application of RDF+ MgSO4(50 kg/ha) resulted in higher values for chlorophyll in leaf and fruit of okra. Among the varieties studied, Arka Anamika had comparatively higher value of chlorophyll content (0.141

mg/gm) while Sadabahar contained lower value of chlorophyll (0.135 mg/gm) in fruit of okra.

Patel *et al.* (2012) studied 10 different cultivars of okra, in which higher chlorophyll content was found in AOL-8-5 (0.56 mg/gm) and lower chlorophyll content was found in AOL-03-1 (0.18 mg/gm).

Merina and Mary (2012) recorded the highest chlorophyll in control (6.05 mg/gm), while the lowest chlorophyll content was observed 150 mM Nacl treatment (2.89 mg/gm).

Rusea *et al.* (2014) observed that in Malaysian okra variety total chlorophyll was higher in mature leaves as 1.56mg/0.1g, and the lowest was in mature fruit as 0.11mg/0.1g.

Rao and Sharma (2013) studied the metabolites of okra after the infection of pest, and reported that okra leaves contained 9.95 mg/gm chlorophyll.

2.4.12 Total carotenoids content

Teow *et al.* (2006) studied β-carotene content in sweet potato genotypes. They reported that Hernandez contained 167 µg/g carotene, while Beauregard contained 92.3 µg/g carotene.

Nair *et al.* (2013) reported that beta carotene (precursor of vitamin A) had potential antioxidant activity. It has important function in photosynthesis, nutrition and protection against photooxidative damage. They observed that Arka Anamika contained 0.624mg/gm carotene.

Arlai *et al.* (2009) observed the rate of carotene increased in organic okra was higher than normal okra, i.e., 10.1 and 4.9 (% wet basis), respectively.

Merina and Mary (2012) studied the effect of gibberellic acid on the counteraction of the Nacl 50, 100 and 150 mm, which induced deleterious effects on

okra leaves in which higher carotenoids content (2.15 mg/gm) was observed in 0 mM concentration of Nacl, while lower carotenoids content (0.12 mg/gm) was observed in treatment with 150 mM concentration.

2.4.13 Peroxidase activity

Nair *et al.* (2013) observed increased activity of peroxidase enzymes throughout fruit development. Peroxidase activity was significantly high at all stages.

Priscila *et al.* (2008) determined activity of peroxidase from the plant organs of tomato cultivar (Micro-Tom) after 104 days of development. The total activities were higher in the stem than in other tissues, whereas the stem exhibited the lowest activity. Staining analysis following gel electrophoresis revealed the existence of four isoenzymes in leaves, three in fruits, but only two in the roots and stems.

Biles *et al.* (2000) reported an increase in exocarp peroxidase activity in musk melon fruits at 5 to 30 days after post pollination, and decreased after 40 to 50 days after post pollination. Total peroxidase activity of the mesocarp was significantly lower than the exocarp in all developmental stages. Mesocarp peroxidase activity decreased consecutively from outer to middle and to the inner tissue at every developmental stage.

2.4.14 Polyphenol Oxidase activity

Khorsheduzzaman *et al.* (2010) studied biochemical basis of resistance to *Leucinodes orbonalis guenee* and their correlation with shoot and fruit borer damage in five selected brinjal genotypes. Results showed that PPO activity was higher in fruit as compared to shoot. Both shoot and fruit from less susceptible genotypes had higher amount of polyphenol oxidase.

Polyphenol oxidase (o-diphenol: oxygen oxidoreductase, EC 1.10.3.1.) had been found in higher amount in plants, and was responsible for enzymatic browning

of raw fruits and vegetables (Mathew and Parpia, 1971). This reaction was important in food preservation and processing, and was generally considered to be an undesirable reaction because of the unpleasant appearance and concomitant development of an off flavour.

2.4.15Catalase activity

Rajaram *et al.* (2013) determined activity of catalase from okra cultivar during the drought stress. Catalase activity was increased in all the parts of the plants to a larger extent under all the treatments in *Abelmoschus esculentus* L. Catalase activity increased in drought stress and with TDM treatments compared with control. Enzyme activity in stress plants treated with TDM showed non significant increase compared with control.

Nair *et al.* (2013) observed less quantity of catalase at the initial stages of development, it increased later on and the values differed significantly in all the samples of okra fruit.

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 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.

2.5.2 Isoenzymes:

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 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, Soltis 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.

Assays of specific activities and electrophoretic separations of multiple forms of peroxides on PAGE in cotton resistant and susceptible genotypes to *Meloidogyne incognita* were conducted six days after inoculation. Specific activities were greater in infected root tissues than in uninfected root and the activities were higher in resistant cultivar. This phenomenon indicated that there was an occurrence of altered metabolism upon infection and differences in enzyme activity between resistant and susceptible cultivars (Noel and McClure, 1977).

Pathmarajah and Eeswara (2005) detected isozymes variability by starch gel electrophoresis from young leaf tissues of seven-day-old seedlings of binjal grown under green house conditions. They studied six enzymes including esterase in 16 brinjal accessions and three cultivars available in Sri Lanka. Unique combinations of isozymes variants of all enzymes assayed were able to differentiate thirteen accessions and two cultivars (79%). Their results suggested that sufficient variability was present in brinjal to allow the use of isozyme analysis as a system for cultivar identification.

Studies on the biochemical basis of five selected brinjal genotypes were carried out by Khorsheduzzaman *et al.* (2010). They recorded that few genotypes had higher amount of polyphenol oxidase (PPO). Significant negative correlation was found between percent infestation (shoot and fruit) with PPO content, whereas it was positively correlated with reducing sugar content.

2.6 MOLECULAR MARKER

2.6.1 Simple sequence repeats (SSR):

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 Agicultural Univesity, Anand during Summer 2012-13. 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. The present physico-chemical properties of the experimental field are presented in Table -3.1.

Table 3.1: Physico-chemical properties of soil of the experimental plot

Sr. No.	Soil properties	Soil depth(0-15 cm)	Method employed
A. Physical properties			
1	Mechanical fraction (%)		
	Coarse sand	0.64	
	Fine sand	82.26	International Pipette Method (Piper, 1966)
	Silt	12.43	
	Clay	5.6	
2	Texture class	Loamy sand	
B. Chemical properties			
1	Organic carbon (%)	0.39	Walkley and Black Method (Jackson, 1973)
2	Available nitrogen ($kg\ ha^{-1}$)	302.1	Alkaline $KMnO_4$ Method (Subbiah and Asija, 1956)
3	Available P_2O_5 ($kg\ ha^{-1}$)	49.38	Olsen's Method (Chopra and Kanwar, 1976)
4	Available K_2O ($kg\ ha^{-1}$)	557.4	Flame Photometric Method (Jackson, 1973)
5	pH (Soil : water,1 : 2.5)	8.04	Blackman's pH meter (Jackson, 1973)
6	EC (dSm^{-1}) (Soil:water,1: 2.5)	0.36	Solubridge Method (Jackson, 1973)

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 10 cultivars of okra

(1) Ankur-40 (2) Arka Anamika (3) GAO -5 (4) GO – 2 (5) Ganesh

(6) Glory (7) Jyoti (8) Nirmala-303 (9) Parbhani kranti (10) Shive.

3.2.3 Layout details

The experimental design randomize complete block design was used, so 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.

3.2.3.1 Field experiment

1. Crop	: Okra
2. Cultivar	: 10 Cultivars
3. Year and season	: *Summer*, 2013
4. Design	: (Randomize complete Block Design)
5. Replication	: 3 (Three)
6. Plot size	: 1.8 X 2.4 m
8. Total number of plots	: 30
9. Spacing	: 60 X 30 cm^2
10. Manures and fertilizers	: N: P: K @ 100: 50: 50 kg/ha

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 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 Date of sowing: 25th February 2013

3.4 Cultural practices

Cultural practices like irrigation, weeding, plant protection measure etc., were carried out according to the recommended agronomical practices of okra crop.

Table 3.2: Cultural practices carried out during experiment

Cultural Practices	1st Time	2nd Time	3rd Time	4th Time
Weeding	07-03-2013	18-03-2013	29-03-2013	12-04-2013
Gap filling	10-03-2013	20-3-2013	-	-
Irrigation	08-03-2013	22-03-2013	3-04-2013	15-04-2013
Plant protection sprays	1-04-2013 Penu-methyl	03-06-2013 Confidor	28-06-2013 Spark	-

3.5 Observations

All the observations with respect to growth parameters, yield attributing characters, plant protection and biochemical changes of fruits at middle picking were recorded. Five plants were selected randomly and tagged from each treatment, for the purpose of recording observations are under.

3.5.1 Growth and yield attributes

3.5.1.1 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.

3.5.1.2Fruit length (cm)

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

3.5.1.4 Fruit diameter (cm)

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

3.5.1.5 Fruit weight (g)

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

3.5.1.6 Fruit Volume (cm^3)

The fruit volume of 5 randomly selected fruits was measured after the harvest. Volume of fruits was 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.

3.6.1.6 Fruit density (g/cm^3)

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

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

3.5.1.7 Fruit yield (q/ha.)

Total yield per plant from each selected plant as well as per plot were recorded and converted into fruit yield quintal per hectare.

3.5.2 Pest damage status

3.5.2.1 Number of larvae of fruit borers

Larvae of fruit borer were counted on fruit by visual observation on the randomly selected five plants.

3.5.2.2 Number of fruit and shoot borers

Larvae of fruit and shoot borer were counted on fruit by visual observation on the randomly selected five plants.

3.5.3 Biochemical attributes

3.5.3.1 Moisture

Moisture content of okra fruits was determined by drying the weighed sample of okra fruit sat 105° C in hot air oven for 5 hours and the loss of weight was expressed as moisture content (A.O.A.C., 2000). Five gram fruit sample from each variety was taken in pre-weighed petriplates and calculate the moisture by the following formula:

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

3.5.3.4 Total carbohydrates

The okra fruit 1 g was homogenized in 2N hydrochloric acid using mortar and pestle and volume was made to 20 ml. The content was refluxed for one hour on boiling water bath at 70˚C. Supernatant was collected and residue was re-extracted twice with 2N HCl. All supernatant were pooled and final volume was made to 50 ml. The extract was used for the estimation of total carbohydrates. Total carbohydrates were estimated by following the method suggested by Dubois *et al*. (1956) with some modifications. Aliquot 0.5 ml was taken and volume was made to 3 ml with the distilled water followed by 0.5 ml distilled phenol and mixed thoroughly. To this, 5.0 ml concentrated sulphuric acid was carefully added at the side of the tube. After the mixing thoroughly the tubes were kept for 30 minutes at room temperature for colour development. The absorbance was measured at 490 nm. The carbohydrate standard range was 100 µg/ml.

$$\text{Carbohydrate}\,(\%) = \text{Graph factor x}\,\frac{\text{Sample reading}}{\text{Weight of sample}}\text{x}\frac{\text{Total volume}}{\text{Taken volume}}\text{x}\,10^{-4}$$

3.5.3.2 Mucilage

The okra fruits were homogenized with five times its weight of water, centrifuged at 4000g for 15 min and the clear, viscous solution decanted. The solution was heated at 70°C for 5 min to inactivate enzymes, and recentrifuged. The mucilage was precipitated with three volumes of ethanol and washed with more ethanol followed by acetone. The cream coloured solid was dried under vacuum (less than 1 Torr at 25°C for 12 h) and weighed (Woolfe *et al.*, 1976).

3.5.3.3 Fiber content

Fibre content was determined by the method developed by Maynard (1970). Fresh 1.0 g okra fruit sample was taken and extracted with petroleum ether to remove fat. After extraction, 200 ml sulphuric acid added into dried sample for 30 minute and then sample was filter. After this, the sample was boiled with 200 ml of NaOH for 30 min. and again filtered the sample and washed with 25 ml of boiling sulphuric acid, 50 ml water and 25 ml alcohol. Then remove the residue and transfer into the pre-weighted dish W1 and dry the residue for 2 h at 130° c (W2). After this ignite the sample for 130° c for 30 min then cool the sample and reweighted(W3).

$$\text{Crude fibre}\,(\%) = \frac{\text{Loss in weight on ignition}\,(\text{W2} - \text{W1}) - (\text{W3} - \text{W1})}{\text{Weight of sample}}\text{x}\,100$$

3.5.3.5 Total soluble sugars

The okra fruit sample (1.0 g) was homogenized in 80 % methanol using mortar and pestle and final volume was made to 10 ml. The content was refluxed for two hour on boiling water bath at 65°C. Supernatant was collected and residue was re-extracted twice with 80 % methanol. All supernatant were combined and final volume

was made to 25 ml. The extract was used for the assay of total soluble sugars and reducing sugars.

Aliquot 0.5 ml was taken and volume made to 3 ml with the distilled water followed by 0.5 ml distilled phenol and mixed thoroughly. To this, 5.0 ml concentrated sulphuric acid was carefully added at the side of the tube. After the mixing thoroughly the tubes were kept for 30 minutes at room temperature for colour development. The absorbance was measured at 490 nm. The content was calculated with the help of a reference curve prepared from D-glucose as standard and expressed as percent. The total soluble sugar standard range was 50-300µg/ml.

$$\text{TSS (\%)} = \text{Graph factor x} \frac{\text{Sample reading}}{\text{Weight of sample}} \text{x} \frac{\text{Total volume}}{\text{Taken volume}} \text{x } 10^{-4}$$

3.5.3.6 Reducing sugars

Reducing sugars content was estimated by the method suggested by Somogyi (1944).

Suitable quantity of aliquot (0.5 ml) was taken and made the final volume 1.0 ml with distilled water. To this 1.0 ml of alkaline copper reagent (4.0 g. copper sulphate, 21.0 g. sodium carbonate, 12.0 g. sodium potassium tartrate, 16.0 g. sodium bicarbonate, 180 g. anhydrous sodium sulphate dissolved in 1000 ml of distilled water) was added and the tubes were incubated in boiling water bath for 10 minutes. The content was cooled and 1.0 ml of arsenomolybdate reagent (50 g. ammonium molybdate, 42 ml concentrated sulphuric acid, 6.0 g. disodium hydrogen arsenate in distilled water made up 1000 ml) was added to each tube. Finally total volume was made up to 10 ml with distilled water. The absorbance was measured at 540 nm. Reducing sugar content was calculated from the standard curve prepared from D-glucose. The reducing sugar standard range was 100 µg/ml.

$$\text{Reducing sugars}(\%) = \text{Graph factor x } \frac{\text{Sample reading}}{\text{Weight of sample}} \text{x} \frac{\text{Total volume}}{\text{Taken volume}} \text{x } 10^{-4}$$

3.5.3.7 Total phenol

The fruit sample (1.0 g) was homogenized in 80% methanol using mortar and pestle and final volume was made to 10 ml. The content was refluxed for two hour on boiling water bath at 65° C. Supernatant was collected and residue was re-extracted twice with 80 % methanol. All supernatant were combined and final volume was made to 25 ml. The extract was used for the assay of total phenol.

Total phenol was estimated by following the method of Bray and Thorpe (1954) with some modifications.

Aliquot 0.5 ml was taken and made the final volume 1.0 ml with distilled water. To this add 0.5 ml of Folin–Ciocalteu reagent and after 3 min 2 ml of 20% Na_2CO_3 was added and the tubes were incubated in boiling water bath for 1 min., cooled it, and made total volume 10 ml with distilled water. The absorbance was measured at 650 nm. Phenol content was calculated from the standard curve prepared from Catechol. The total phenol standard range was 100 µg/ml.

$$\text{Phenol } (\%) = \text{Graph factor x } \frac{\text{Sample reading}}{\text{Weight of sample}} \text{x} \frac{\text{Total volume}}{\text{Taken volume}} \text{x } 10^{-4}$$

3.5.3.8 True protein

Protein content was determined by the method developed by Lowry *et al.* (1951).

The fruit sample (1 gm) was weighed and homogenized in five ml 0.1 N NaOH and filtered through Whatman No.1 filter paper. The sample extracts 0.2 ml was taken and made to 3.0 ml volume with distilled water. 5 ml of alkaline copper solution (50 ml 2% Na_2CO_3 in 0.1 N NaOH + 1 ml 0.5 % $CuSO_4$ in 10 % Sodium Potassium tartrate) was added. The content was allowed to stand for 10 minutes at

room temperature followed by addition of 0.5 ml solution FolinCiocalteu reagent (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.

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

3.5.3.9 Total Antioxidant activity

One gram of sample was extracted it in 10 ml of 60% aquous methanol containing 0.1% HCL. It was shaked for 4 hours at room temperature and centrifuged at 10000rpm/15min at 10 ºC. The slolution was filtered and store it in freeze. Aliquot 1ml of extract and mix it with 3ml FRAP reagent. Incubate 10 minute at room temperature. The absorbance was read at 593nm.

$$\text{Antioxidant activity (\%)} = \frac{\text{Abs control} - \text{Abs sample}}{\text{Abs control}} x\ 100$$

3.5.3.10 Ascorbic acid

Ascorbic acid content was estimated by 2, 4-Dinitro phenyl hydrazine (DNPH) method as described by Bhatnagar *et al.*, 2007.

One gram okra fruit sample from each stages were extracted in 20 ml of 3 % trichloro acetic acid (TCA). A pinch of activated charcoal was added during extraction. The sample was homogenized with the help of mortar and pestle. Then collected the supernatant by filtration. 1.0 ml of supernatant aliquots was taken in each test tube. To this 1 ml of 2 % DNPH (prepared in 9 N H_2SO_4) and 0.5 ml 10 % thiourea was added. All test tubes including blank were incubated for 5 hrs at 37^0 C. After all tubes were placed in ice bath and 5 ml of 85 % H_2SO_4 was added. The tubes were then allowed to stand for 2 hrs at room temperature and the

absorbance read at 540 nm. Standard ascorbic acid was used in the concentration range of 5-30 µg to serve as a reference graph.

The amount of ascorbic acid in sample was calculated as follows

Ascorbic acid (mg/100g)

$$= \text{Graph Factor X Sample reading X } \frac{\text{Total volume}}{\text{Taken volume}} \text{ X } \frac{1}{\text{Weight of sample}} \text{ X } 10^{-4}$$

3.5.3.11 Chlorophyll

Chlorophyll was estimated by method described by Hiscox and Israelstam (1979). Hundred mg fresh fruits were cut into small pieces and kept in dimethyl sulfoxide (DMSO) containing tube over night. The extract was filtered through Whatman No.1 filter paper. Filtrate was collected and volume made to 10 ml with DMSO. Absorbance was measured in spectrophotometer at 645 nm and 663 nm for determination of total chlorophyll, and content was calculated by following equations:

$$\text{Chlorophyll a (mg/g fresh tissue)} = \frac{12.2\ (A_{663}) - 2.69\ (A_{645})}{\text{a x W x 1000}} \text{x V}$$

$$\text{Chlorophyll b (mg/g fresh tissue)} = \frac{22.9\ (A_{645}) - 4.68\ (A_{663})}{\text{a x W x 1000}} \text{x V}$$

$$\text{Total Chlorophyll (mg/g fresh tissue)} = \frac{20.2\ (A_{645}) + 8.02\ (A_{663})}{\text{a x W x 1000}} \text{x V}$$

Where A_{663} = Absorbance at 663 nm

A_{645} = Absorbance at 645 nm

a = Length of light path in the cell (usually 10 mm)

V = Volume of the extract in ml

W = Fresh weight of the sample in gm

3.5.3.12 Carotenoids

Carotene was estimated by method described by Nagata and Yamashita (1992).

Fresh 1.0 g okra fruit sample was taken and extracted with Acetone: Hexane (4:6) at once. The samples were homogenized with mortar and pestle. Supernatant were collected. Optical density of supernatant was measured at 663, 645, 505 and 453 nm with the help of spectrophotometer. From these values, the content of carotene was analyzed by following equations:

carotene (mg/100g) = $0.216A_{663} - 1.22A_{645} - 0.304A_{505} + 0.452A_{453}$

A_{663}, A_{645}, A_{505} and A_{453} were absorbance at 663nm, 645nm, 505nm and 453 nm respectively.

3.5.4 Enzyme activities

3.5.4.1 Peroxidase (EC 1.11.1.7)

Substrate preparation

Five ml of 6 % H_2O_2 solution diluted to 100 ml with water. Take one ml from this and again diluted to 100 ml with 0.01 M Sodium phosphate buffer (pH 6.0). Six ml of this solution was mixed with 0.05 ml dye solution and use as a substrate.

Enzyme extraction

One gram of okra fruit sample from each variety was homogenized in a pre-chilled mortar and pestle under ice cold condition in 5 ml of extraction buffer, containing 0.1 M sodium phosphate buffer (pH 7.2) with the addition of pinch of polyvinyl pyrolidonex (PVP). The homogenate were centrifuged at 10,000 rpm for 20 minutes and the supernatant used for the assay (Guibault, 1976).

Peroxidase enzyme activity was determined in the supernatants of centrifuged homogenate by measuring the increase in absorbance at 460 nm in a reaction mixture containing three ml of substrate and 0.1ml of enzyme extract for every 10 seconds till 1 minute. For blank substrate was not used.

Enzyme activity = Change in O.D. /min /g of fresh tissue

3.5.4.2 Polyphenol oxidase (EC 1.14.18.1)

Substrate preparation

Take two ml of 0.01 M Catechol in 0.01 M Sodium phosphate buffer (pH 6.0) and use as a substrate.

Enzyme extraction

One gram of okra fruit sample was homogenized in a pre-chilled mortar and pestle under ice cold condition in 2 ml of extraction buffer, containing 0.1 M sodium phosphate buffer (pH 7.2) with the addition of pinch of polyvinyl pyrollidone (PVP). The homogenate were centrifuged at 10,000 rpm for 20 minutes and the supernatant used for the assay (Malik and Singh, 1980).

Polyphenol oxidase enzyme activity was determined in the supernatants of centrifuged homogenate by measuring the increase in absorbance at 490 nm in a reaction mixture containing three ml of substrate and 0.01ml of enzyme extract for every 10 seconds till 1 minute.

Enzyme activity = Change in O.D. /min /g of fresh tissue

3.5.4.3 Catalase (EC 1.11.1.6)

Substrate preparation

Take the 3ml of 0.1M phosphate buffer with 2ml of 0.7N H_2SO_4 (pH 7.0) and use as a substrate.

Enzyme extraction

One gram of okra fruit sample was homogenized in a pre-chilled mortar and pestle under ice cold condition in 2 ml of extraction buffer, containing 0.1 M sodium phosphate buffer (pH 7.0) with the addition of pinch of polyvinyl

pyrollidone (PVP). The homogenates were centrifuged at 15,000 rpm for 20 minutes and the supernatant used for the assay (Luck, 1974).

Enzyme activity = Change in O.D. /min /g of fresh tissue

3.5.5 SDS PAGE

Okra fruit 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.

Reagents/chemicals for electrophoresis

a) Stock acrylamide solution (30%)

29.2 g Acrylamide

0.8 g N, N' – Methylene bis acrylamide.

Final volume made up to 100 ml with double distilled water.

b) Stock 1.5 M Tris-HCL

Tris buffer (18.219 g) was dissolved in 70 ml double distilled water, pH was then adjusted to 8.8 with HCl and final volume was made up to 100ml.

c) Stock 0.5 M Tris-HCl

Tris buffer (6.05 g) was dissolved in 70 ml double distilled water, pH was then adjusted to 6.8 and final volume was made up to 100 ml.

d) Electrode buffer

Tris buffer (3 g) and Glycine (14.4 g) were dissolved in distilled water and volume was adjusted to 1000 ml. For SDS-PAGE 10 ml of 10% SDS was added and then finally volume was made up to 1000 ml.

e) 10 % APS (Ammonium per sulphate)

APS (100 mg) was dissolved in 1 ml of distilled water. Prepared fresh at the time of gel casting.

f) TEMED (N, N, N' N'- Tetra methylethelendiamine) –

Fresh from the refrigerator.

g) 10 % SDS (Sodium Dodecyl Sulphate)

SDS (10 g) was dissolved in 100 ml of distilled water.

h) Gel loading dye

Tris buffer (6.8 pH) - 750 µl

Glycerol - 1 ml

1% Bromophenol blue - 500 µl

Make up the volume 5 ml with distilled water.

For SDS-PAGE 1 ml (10% SDS) was added with above solutions and final volume was made up to 5 ml.

i) Staining Dye for protein

Commassie brilliant blue R-250 (0.1 g) was dissolved in 100 ml solution of Methanol (40): Acetic acid (10): Water (50).

j) Destaining solution

It was prepared by mixing Methanol, Acetic acid and water in the ratio of 40 : 10 : 50.

Table 3.3: Preparation of gel for protein

Reagents	12% Running gel (15 ml)	5% Stacking gel (5 ml)
Double Distilled Water	4.9 ml	3.435 ml
30% Acrylamide	6.0 ml	830 µl
Tris buffer pH 8.8	3.8 ml	630 µl
10% SDS Solution	150 µl	50 µl
10% APS	150 µl	50 µl
TEMED	8 µl	5 µl

3.5.6 Isozymespattern of enzymes

A. **Peroxidase**

B. **Polyphenol oxidase**

C. **Superoxide dismutase**

3.5.6.1 Extraction of Enzymes for Electrophoresis

One gram of okra fruit 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 as described in 3.5.3.8.

a) Preparation of Stock Solution for PAGE

a) Acrylamide/ Bis-Acrylamide stock solution (30%)

Acrylamide - 29.2 g

Bis-Acrylamide (N, N' – Methylene bis acrylamide) - 0.8 g

Final volume made up to 100 ml with double distilled water and stored at 4^0 C in a brown bottle.

b) Tris-HCL buffer stock, 1.5 M (pH 8.8)

Tris buffer (18.219 g) was dissolved in 70 ml double distilled water, pH was then adjusted to 8.8 with HCl and final volume was made up to 100ml.

c) Tris-HCL buffer stock, 0.5 M (pH 6.8)

Tris buffer (6.05 g) was dissolved in 70 ml double distilled water, pH was then adjusted to 6.8 and final volume was made up to 100 ml.

d) Polymerising agents

i. 10 % Ammonium persulphate (APS)

Ammonium persulphate - 100 mg

Distilled water - 1 ml

The solution was made fresh before use.

ii. TEMED (N, N, N, N- Tetra methylethelendiamine) – Fresh from the refrigerator.

e) Electrode buffer

Tris buffer (3 g) and Glycine (14.4 gm) were dissolved in distilled water and volume was adjusted to 1000 ml.

f) Gel loading Dye

Bromophenol Blue (BPB) - 0.5 % (w/v solution in distilled water) –500 mg
Glycerol - 10 % - 10 ml
Volume made up to 100 ml.

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

3.5.6.2 Procedure

a) Peroxidase Isozymes

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 (Sadasivam and Manickam, 2008).

b) 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. (Sadasivam and Manickam, 2008).

c) Superoxide dismutase (SOD)

The gel was soaked in 50 mM sodium phosphate buffer pH 7.8 containing 0.24 mM NBT and 28 mM riboflavin for 20 minutes in the dark

followed by immersing in 50 mM sodium phosphate buffer pH 7.8 containing 28 mM TEMED. Then the gel was exposed to a light source at room temperature until opaque band appeared on blue background. Different isoforms were differentiated by performing activity staining in the gels previously incubated for 20 minutes at 25 °C in 50 M sodium phosphate pH 7.8 containing 5 mM H_2O_2 (Sadasivam and Manickam, 2008).

ISOLATION AND PURIFICATION OF GENOMIC DNA:

3.6 Genomic DNA Extraction:

Extraction of DNA from seedlings was done using modified CetylTrimethyl Ammonium Bromide (CTAB) method (Murray and Thompson, 1980) with some minor modifications.

3.6.1. Preparation of stock solutions for DNA extraction

The reagents and buffers for DNA isolation were prepared as per Sambrook *et al.*, (1989). The composition and procedure for preparation of various stock solutions and buffers are described below.

Preparation of stock solutions for DNA extraction and electrophoresis

1. **1 M Tris HCl (pH 8.0) 100 ml**
    a. 12.11 g Tris base (Himedia) was dissolved in 80 ml distilled water. The pH was adjusted to 8.0 by adding concentrated HCl and the total volume was adjusted to 100 ml. It was dispensed into reagent bottle and sterilized by autoclaving.
2. **0.5M EDTA (pH 8.0) 100 ml**
    a. 18.60 g EDTA di Sodium salt (Amresco) was Dissolved in 80 ml distilled water. The pH was adjusted to 8.0 by adding NaOH pellets.

The total volume was adjusted to 100 ml and dispensed into a reagent bottle and sterilized by autoclaving.

3. **3.5M NaCl 100 ml**
    a. 29.22 g NaCl (Himedia) was taken in to beaker; 50 ml of distilled water was added and mixed well. When the salts got completely dissolved, the final volume was adjusted to 100 ml. It was dispensed into a reagent bottle and sterilized by autoclaving.
4. **80% Ethanol 100 ml**
    a. 80 ml of ethanol was taken and 20 ml of distilled water was added, mixed well and dispensed into a reagent bottle and stored at 4ºC.
5. **Chlorophorm : Isoamyl alcohol (24 : 1) 100 ml**
    a. 96 ml of chloroform (Amresco) and 4 ml of isoamyl alcohol (Amresco) were measured, mixed well and stored in a reagent bottle at room temperature.
6. **Ethidium bromide (10 mg/ml) 1.0 ml**

10 mg Ethidium Bromide (Amresco) was added to 1.0 ml of distilled water and it was kept on magnetic stirrer to ensure that the dye has dissolved completely. It was dispensed into an amber colored eppendorf tube and stored at 4ºC.

Preparation of buffers for DNA extraction:-

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 10 mM TrisHCl (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.

3.6.2 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 mMTris-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.

3.6.3. 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 20 ng/µl working solution was prepared from the stock solution of the isolated DNAs.

3.6.3.1. 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.

Table 3.5: Quantification and Preparation of working solution of 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	Ankur-40	982.7	1.84	2.0	98.0
2	Arka Anamika	825.7	1.91	2.4	97.6
3	GAO -5	721.4	1.82	2.8	97.2
4	GO – 2	543.9	1.90	3.7	96.3
5	Ganesh	1098.2	1.96	1.8	98.2
6	Glory	792.6	2.01	2.5	97.5
7	Jyoti	873.7	2.02	2.3	97.7
8	Nirmala-303	697.9	1.93	2.9	97.1
9	Parbhani Kranti	682.2	1.89	2.9	97.1
10	Shive	738.4	1.88	2.7	97.3

3.7 SIMPLE SEQUENCE REPEATS (SSR)

The genomic DNA was amplified using primers listed in table 3.8. 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 (MIcroSAtellite search tool) (Thiel *et al.,* 2003). The MISA 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.

3.7.1 Master mix preparation

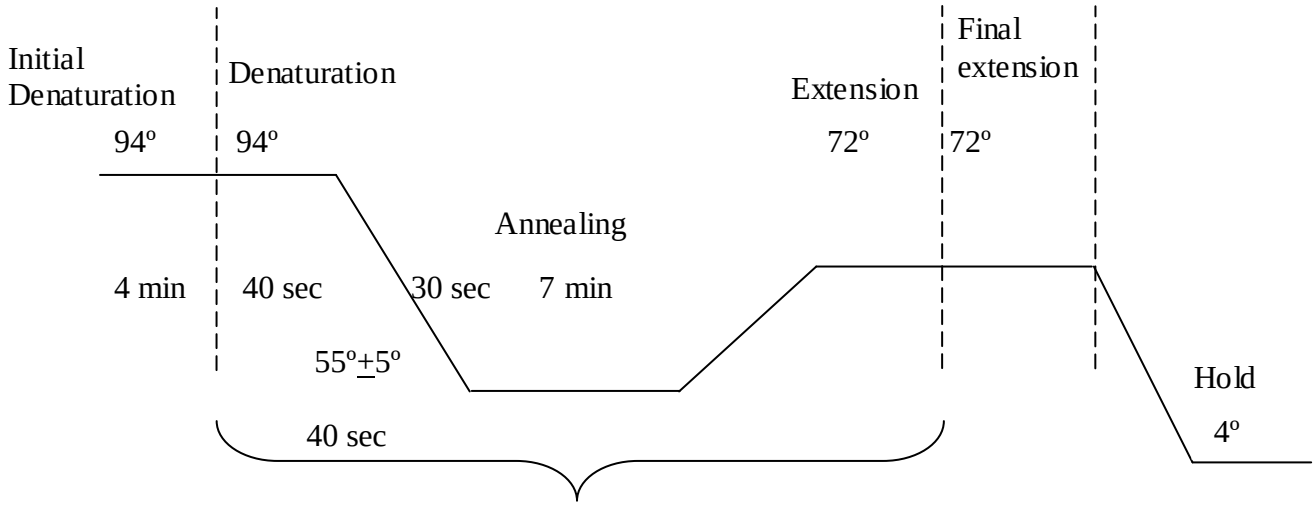
All the PCR reactions were carried out in 200 µl thin walled PCR tubes. Cocktail for PCR reaction was prepared by adding 10X Standard Taq Buffer with $MgCl_2$ (BioLabs, UK) followed by forward and reverse primers, dNTPs, Taq DNA polymerase (BioLabs, UK) and template DNA. The reagents were mixed by gently 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: 3.6 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

Table 3.7: List of SSR Primers

Sr. No	Markers	F/R	Primer sequences
1	OK-1	F	CAGGGATAACAGAGGAAATCT
		R	TAGAGAGCGGTGCTACTGTAA
2	OK-2	F	TGGTCTTCAGTGTAAGGTTTC
		R	CCCACACTTTAACATCTAACG
3	OK-3	F	GAAAAGTACCGAGGAGAGGTA
		R	AGCAATTAGCCCTACAAGAAG
4	OK-4	F	GACACTAGCTGAAGAACATGG
		R	GATTTTGCATCAGAATCAGAC
5	OK-6	F	TTTCACTTCATCCAAATGACT
		R	TTCAGAATGGTAAACAAAACG
6	OK-7	F	AACGAAATCACTAATGCCATA
		R	ACCAATTTATCTTCCCTGAAC
7	OK-9	F	GAAGTCACTGGAGGTCCTACT
		R	TAATGCCACAATATCCTTGTC
8	OK-12	F	TAATGCCACAATATCCTTGTC
		R	GAAGTCACTGGAGGTCCTACT



α

40 cycles

Figure 3.1: PCR reaction conditions for SSR primers

3.7.2 Electrophoresis of amplified products

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

3.7.2.1 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 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).

3.8 STATISTICAL ANALYSIS:

Clear and distinct bands amplified by SSR primer were scored for the presence and absence of the corresponding band among the genotypes. The scores 1 and 0 indicate the presence or absence of bands, respectively. The softwares used for the analysis of the scored data were NTSYSpc version 2.02 (Rholf 1994).

3.8.1 Rm (Relative Mobility) Value:

The relative mobility (Rm) of each band was measured in each zymogram for every cultivar tested using the following equation (Eeswara and Peiris, 2001).

$$Rm = \frac{\text{Distance migrated by the enzyme band}}{\text{Distance migrated by the dye marker}}$$

3.8.2 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 okra cultivars were expressed in the form of dendrograms and genetic similarity matrix.

3.8.3 Cophenetic correlation and Mantel tests

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 correspondence of SSR, based on similarity coefficient matrices was tested using cophenetic correlation analysis and Mantel matrix correspondence test. The Mantel matrix correspondence test was carried out using the MXCOMP function in the NTSYSpc version 2.02.

3.8.4 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 %

- Polymorphism Information content (PIC) was calculated according to formula described by Bootstein *et al.*, (1980) and (Anderson *et al.*, 1993).

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

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

Marker Index (MI) = EMR X DI $(av)_p$

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

EMR= $n_p(n_p/n)$

Wheren = $n_p+n_{np,}$ n_p = polymorphic loci, n_{np} = non-polymorphic loci and $DI(av)_p$ = average Diversity Index of Polymorphic loci = $\sum DI_n/n_p$ (DI = PIC), $\sum DI_n$ = $\sum$PIC.

All the above mentioned variables were calculated individually for all the three markers as well as the combined values for SSR was calculated for testing the combined ability of the markers for genetic diversity assessment.

3.8.5A summary of the statistics used in analysis of SSR markers

The analysis was performed using GenALEx 6.3 software and calculated manually in Microsoft *Excel* 2007 (Peakall and Smouse 2006).

- Allele Frequency (Codominant Data)

$$\boldsymbol{FreqAllele\_x=} \frac{Nxx+Nx}{2N}$$

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 (Codominant Data)

$$\text{Ho} = \frac{No.\_of\_Hets}{\text{N}}$$

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

- Expected Heterozygosity or Genetic Diversity (Codominant Data)

$$He = 1 - \sum p_i^2$$

Calculated on a single locus basis. GenAlEx also provides the arithmetic mean across loci. Here, pi is the frequency of the i^{th} allele.

CHAPTER IV
RESULTS AND DISCUSSION

Okra (*Abelmoschus esculentus* L.) is an important vegetable crop which is widely distributed from Africa to Asia. This crop also prevalently known as bhendi. The field experiment was conducted at Main vegetable research station, Anand agricultural university, Anand during Summer 2013 to study "Morphological, Biochemical and Molecular characterization of Okra Cultivars (*Abelmoschus esculentus* L.)".The results are presented below.

4.1 Growth and yield 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 76.52 – 101.87 cm (Table 4.1, &Fig.4.1). Significantly the highest plant height was registered for GAO-5 (101.87 cm).Whereas significantly lower plant height was recorded for cv. Glory (76.52 cm) which was followed by Arka Anamika (78.66 cm) and Ganesh (78.83cm).

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 (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 GAO-5 (13.70cm), which was at par with GO-2 (13.56 cm), while significantly lower fruit length was observed for Glory (10.17 cm).Non significant differences were recorded among Shive, Parbhani kranti, Ganesh, Nirmala-303, Ankur-40, Jyoti, Arka Anamika and Glory.

Kabir and Pillu (2011) found that the okra fruit length was maximum 11.83cm 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 2.30 – 2.83 cm (Table 4.1, &Fig.4.2). The maximum fruit girth recorded for GAO-5, which was followed by GO-2 (2.66 cm), Parbhani kranti (2.65 cm), Nirmala-303 (2.62 cm) and Jyoti (2.62 cm). The minimum fruit girth was observed for Glory (2.30cm), and followed by Arka Anamika (2.39 cm), Ankur-40 (2.46 cm) and Ganesh (2.47 cm).

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 (g)

The highest fruit weight was recorded for GAO-5 (12.37g) followed by GO-2 (11.79g). Minimum fruit weight was recorded for Glory (9.48g), and it was statistically at par with Arka Anamika (9.64 g) and Ganesh (10.14 g) (Table 4.1, & Fig. 4.2).

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

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.4.2. Significantly higher fruit volume was registered for GAO-5 (9.28 cm^3), which was statistically at par with Parbhani kranti (8.70 cm^3), Jyoti (8.68 cm^3), Nirmala-303 (8.67 cm^3), Shive (8.59 cm^3) and GO-2 (8.21 cm^3). Whereas, significantly lower fruit volume was recorded for Glory (7.41 cm^3), which significantly at par with Arka Anamika (7.49 cm^3) and Ankur-40 (8.07 cm^3).

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. 4.2. The maximum and minimum fruit density was recorded for GO-2 (1.44g/cm^3). and Shive (1.20g/cm^3), respectively.

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

4.1.7 Fruit Yield (q/ha)

From perusal of experiment results reveals was found that okra fruit yield ranged from 72.43q/ha to 85.89 q/ha (Table 4.1, & Fig.4.1). Significantly higher fruit yield was recorded for GAO-5 (85.89 q/ha), which was statistically at par with GO-2 (84.41 q/ha) and Nirmala-303 (84.19 q/ha). While, the least fruit yield was recorded for Glory (72.43 q/ha), which was found to be significantly at par with Ganesh and Arka Anamika.

Mandal *et al.* (2006) evaluate seven okra cultivars *viz.* ArkaAbhay, Arka Anamika, D-1-87-5, D-1-87-16, HRB-55, KS-312 and PusaSawani for their fruit yield, and 104.54 q/ha, fruit yield with was recorded in D-1-87-5, followed by D-1-87-16 (102.06 q/ha), ArkaAbhay (96.97 q/ha) and ArkaAnamika (96.51 q/ha). The least fruit yield (86.84 q/ha) was observed with Pusasawani. Tapankumar and PradyumnaTripathi (2009) recorded maximum fruit yield with variety NOH 15 (107.98 q/ha) and minimum fruit yield was with Panchali (74.30 q/ha).

Table 4.1:- Plant height, fruit length, fruit yield, fruit weight, fruit girth and fruit volume of different cultivar of okra.

Cultivar	Plant height (cm)	Fruit length (cm)	Fruit girth(cm)	Fruit weight(g)	Fruit volume(cm^3)	Fruit density (g/cm^3)	Yield (q/ha)
Ankur-40	82.48	12.00	2.46	10.37	8.07	1.29	75.42
Arka Anamika	78.66	11.95	2.39	9.64	7.49	1.29	73.53
GAO -5	101.87	13.70	2.83	12.37	9.28	1.34	85.89
GO – 2	98.20	13.56	2.66	11.79	8.21	1.44	84.41
Ganesh	78.83	12.003	2.47	10.14	8.46	1.20	73.72
Glory	76.52	10.17	2.30	9.48	7.41	1.30	72.43
Jyoti	89.21	12.69	2.62	10.88	8.68	1.25	77.75
Nirmala-303	93.41	12.95	2.62	11.13	8.67	1.29	84.19
Parbhani kranti	93.08	12.84	2.65	11.66	8.70	1.34	80.67
Shive	89.40	12.69	2.47	10.35	8.59	1.20	76.31
S.Em.±	0.98	0.41	0.11	0.18	0.32	0.057	0.813
C.D.@5%	2.91	1.21	0.33	0.54	0.95	0.170	2.416
CV%	1.92	5.72	7.48	2.93	6.60	7.664	1.797

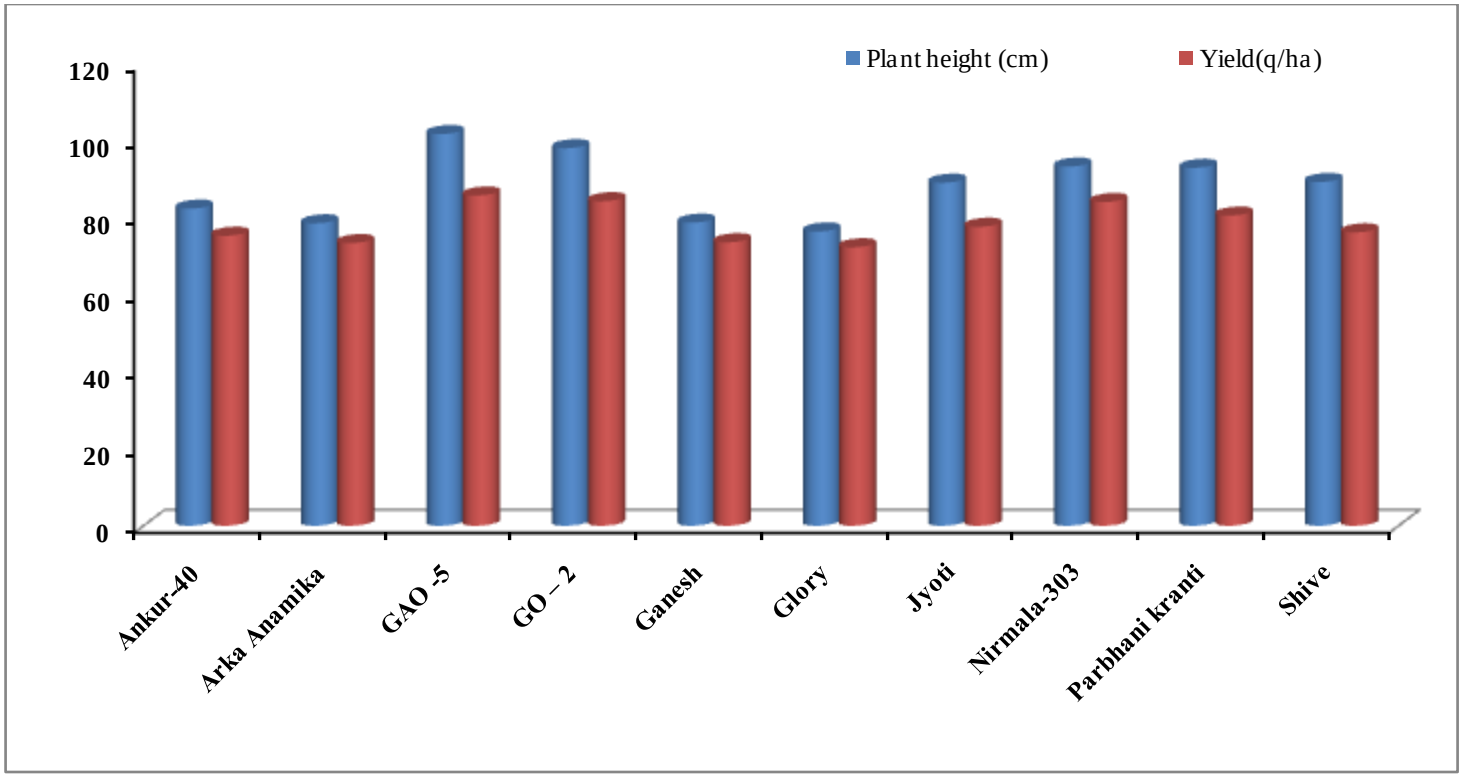


Fig. 4.1:- Plant height and fruit yield of different cultivars of okra.

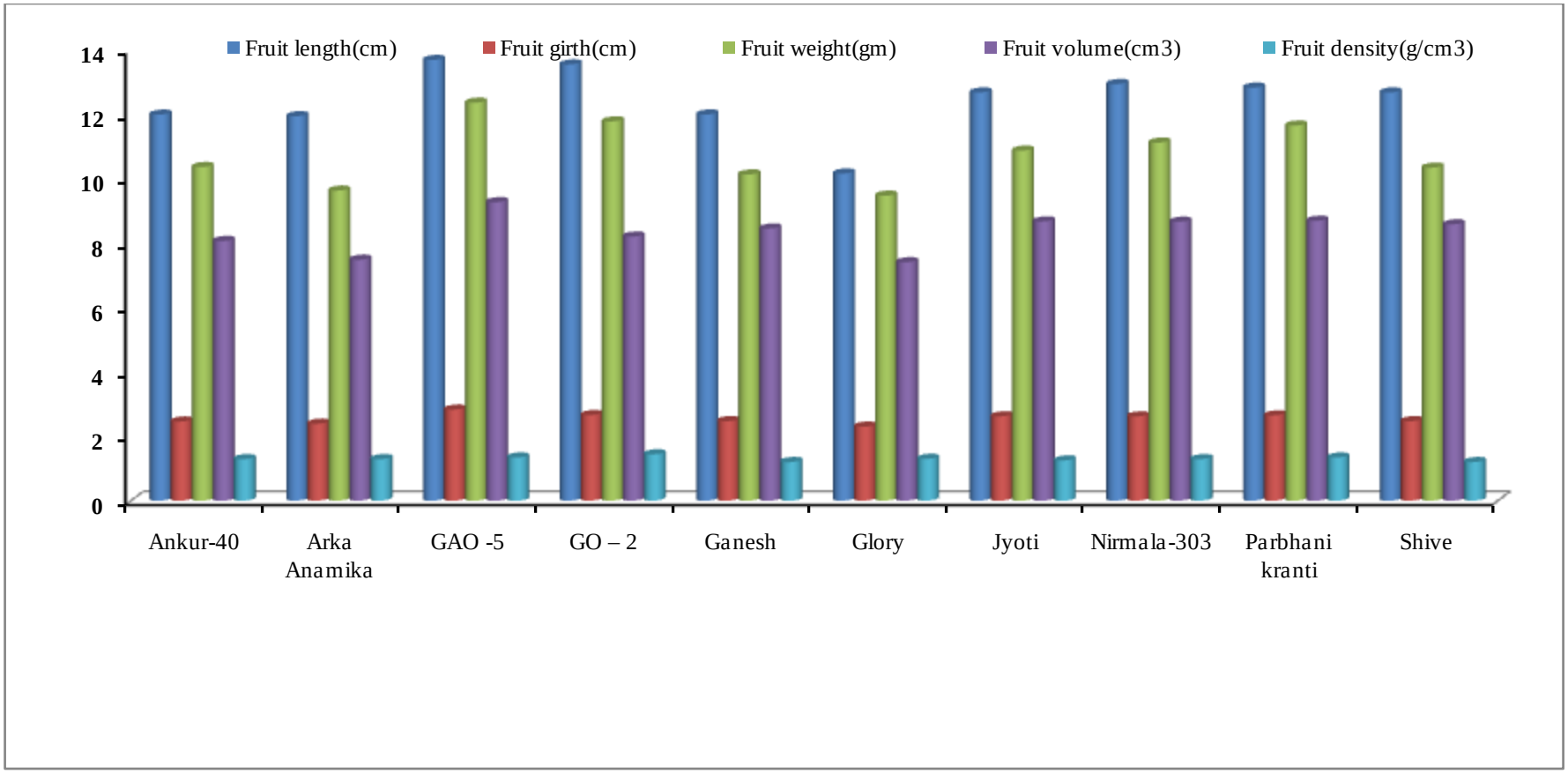


Fig. 4.2:- Fruit length, fruit girth, fruit weight, fruit volume and fruit density of different cultivars of okra

4.2 Pest damage status

There are many factors affecting productivity of okra. One of them losses caused by insect pests. The crop is affected by number of insect pest, mites and nematodes during different growth stages. Of these pests, shoot and fruit borer [*Eariasvittella* (Fabricius)] is the most destructive pests causing economic damage to the crop at all the growth stages.

4.2.1 Fruit and shoot borer

Fruit and shoot damage caused by *E. vittella* was studied in different cultivars of okra at 35 DAS (Table 4.2).

The significance differences were observed for fruit and shoot borer among all okra cultivar tested. At 35 DAS cv. GAO-5 (1.50%) recorded significantly lower fruit and shoot damage as compared to rest of the cultivars screened. Nirmala-303 was the next one cultivar with respect to less fruit and shoot damage, GAO-5 was statistically at par with Nirmala-303 (1.58%) and GO-2 (2.93 %).

The result of present investigations are in agreement with the findings of Vyas and Patel (1991), who observed wide range of okra fruit damage by fruit and shoot borer. Aziz *et al.* (2012) screened the different cultivars of okra, in which the lowest shoot damage was found in Diksha (8.17%), while the highest fruit damage was found in Parbhani kranti (14.69%).

Mandal and his colleagues (2006) screened the relative susceptibility of seven okra cultivars *viz,* ArkaAbhay, ArkaAnamika, D-1-87-5, D-1-87-16, HRB-55, KS-312 and PusaSawani against *E. vittella* under field conditions, and reported that the minimum shoot damage (8.7%) was recorded in D-1-87-5, which was statistically at par with ArkaAnamika (9.4%), whereas, the maximum shoot damage (11.9%) was observed in PusaSawani.

4.2.2 Fruit borer

Fruit borer population was recorded in different genotypes of okra at 60 days after sowing and data are narrated in Table 4.2.

The data on per cent fruit damage indicated that all the cultivars differed significantly from each other. Genotype GAO-5(2.14%) recorded significantly lower fruit damage as compared to rest of the cultivars screened. Genotype Nirmala-303 was also less affected by fruit damage. However, genotype GAO-5 was statistically at par with Nirmala-303 (2.26 %) and GO-2 (4.18%).

Aziz *et al.* (2012) studied different cultivar of okra in which the lowest fruit damage found in Diksha (8.17%), while the highest fruit infestation was found in Parbhani kranti (18.93%).

Thus, out of ten cultivars screened, genotype GAO-5, Nirmala-303 and GO-2 recorded significantly lower per cent shoot as well as fruit damage, there by could be considered as less susceptible, whereas Glory, Arka Anamika, Ganesh and Ankur-40 could be considered as susceptible to *E. vittella*. Cultivar GAO-5 had lower moisture (73.55%) and higher fibrous (1.067 mg/g), which would have provided tolerance against *E. vittella* infestation.

Table 4.2: Fruit borer and fruit and shoot borer in different okra cultivars

Cultivar	Fruit damage (%)	
	Fruit & shoot Borer 35 DAS	Fruit Borer 60 DAS
Ankur-40	16.31[c] (7.90)	19.61[c] (11.28)
Arka Anamika	25.79[e] (19.0)	31.34[e] (27.14)
GAO -5	7.03[a] (1.50)	8.41[c] (2.14)
GO – 2	9.80[b] (2.93)	11.74[b] (4.18)
Ganesh	20.61[d] (12.45)	24.89[d] (17.78)
Glory	25.86[e] (19.07)	31.41[e] (27.25)
Jyoti	15.15[c] (6.85)	18.20[c] (9.78)
Nirmala-303	7.21[a] (1.58)	8.63[a] (2.26)
Parbhani Kranti	14.69[c] (6.46)	17.64[c] (9.23)
Shive	16.07[c] (7.69)	19.32[c] (10.98)
S.Em.±	0.84	1.04
C.D.@5%	2.50	3.103
CV%	9.19	9.4613

(1) Figures in parentheses are retransformed values; those outside are arc sin transformed values

(2) Treatment means with letter(s) in common are not significant at 0.05 % level of probability respective column

4.3 Biochemical and molecular atributes

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 fruits of different okra cultivars and presented in Table 4.3. Moisture content ranged from 73.55 to 89.66 percent. Significantly higher moisture content was recorded for Glory (89.66%) which was statistically at par with Arka Anamika (88.10%). Significantly the lowest moisture content was recorded for GAO-5 (73.55%). Moisture content was at par for Shive, Jyoti and Parbhani kranti.

Patel *et al.* (2012) studied 10 different cultivars of okra, in which higher moisture content was found in AOL-8-10 (90.41%) and lower moisture content was found in AOL-05-1 (85.33%).

El Balla *et al.* (2011) evaluated moisture content from different cultivars of okra seeds and found that the moisture ranged between 29.88 - 53.60 percent.

4.3.2 Total Carbohydrates

Carbohydrates serve organisms as energy sources and as essential structural components, in addition, part of the structure of nucleic acids, which contains genetic information, also consists of carbohydrate.

The result of present study indicated that carbohydrate ranged between 6.77 - 9.03 percent (Table 4.3, & Fig.4.3). Significantly the highest carbohydrate content was observed in GAO-5 9.03 percent. While significantly the least carbohydrate content was recorded for cultivar Glory 6.77% which was followed by Arka Anamika (7.23%), Jyoti (7.30 %) and Ankur-40 (7.33%).(Table 4.1).

Rusea *et al.* (2014) reported the highest carbohydrate in the mature fruit (11.17%) followed by leaf at (8.83%) and young fruit (7.05%) in okra.

4.3.3 Mucilage

Mucilage reduces oxygen diffusion to the seed and thus has a role in regulating seed dormancy.

Mucilage of okra fruit ranged from 188 – 246 mg/100g (Table 4.3).The maximum mucilage content was recorded for GAO-5, which was followed by GO-2 (237.67 mg/100g) and Nirmala-303 (226.67 mg/100g). The minimum mucilage content was recorded for Glory (188 mg/100g), which was at par with Arka Anamika (206 mg/100 g), Ganesh (211 mg/100g) and Ankur-40 (214.33 mg/100g).

Earlier studies by Woolfe *et al.* (1977) also showed that okra fruit contained 16 g mucilage/kg.

Table 4.3:- Biochemical attributes of moisture, total carbohydrate, mucilage, fiber, total soluble sugar, reducing sugar and nonreducing sugar of okra fruit

Cultivar	Moisture (%)	Total carbohydrates (%)	Mucilage (mg/100g)	Crude fiber (mg/g)	Total soluble sugars (%)	Reducing sugars(%)	Non reducing sugars(%)
Ankur-40	84.60	7.33	214.33	0.467	2.64	0.49	2.14
Arka Anamika	88.10	7.23	206.00	0.433	2.84	0.55	2.31
GAO -5	73.55	9.03	246.07	1.067	2.31	0.44	1.81
GO – 2	75.56	8.37	237.67	1.000	2.57	0.50	2.03
Ganesh	85.06	8.21	211.00	0.467	2.77	0.53	2.28
Glory	89.66	6.77	188.00	0.433	2.90	0.55	2.36
Jyoti	82.03	7.30	223.67	0.700	2.73	0.52	2.11
Nirmala-303	76.50	8.21	226.67	1.000	2.62	0.51	2.17
Parbhani Kranti	80.66	8.14	223.67	0.767	2.57	0.50	2.13
Shive	82.65	7.61	219.33	0.633	2.72	0.52	2.17
S.Em.±	0.67	0.52	5.94	0.116	0.07	0.01	0.07
C.D.@5%	1.99	1.54	17.66	0.344	0.20	0.04	0.22
CV%	1.42	11.46	4.69	28.75	4.46	4.65	5.86

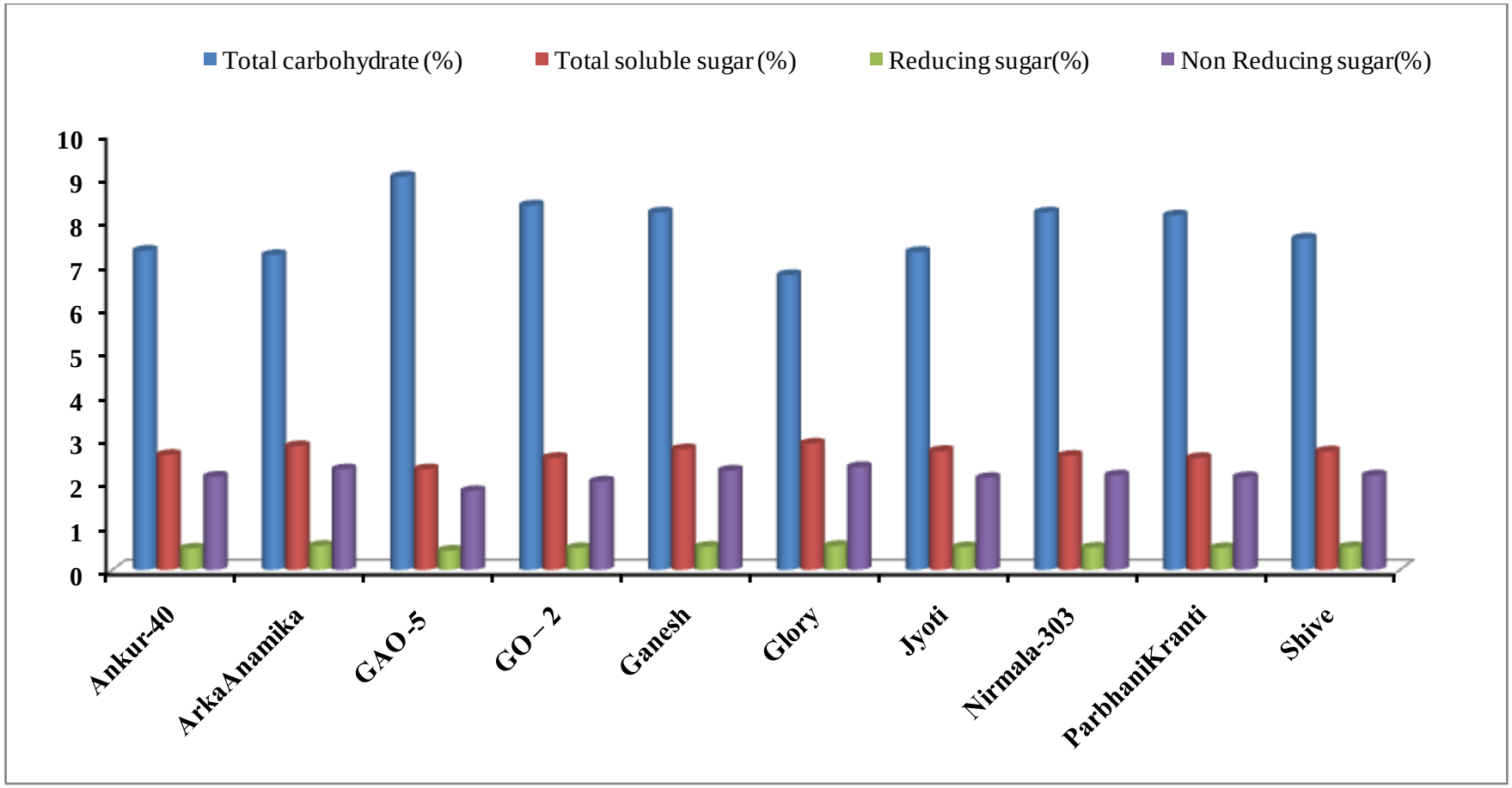


Fig. 4.3:- Total carbohydrate, total soluble sugar, reducing sugar and non reducing sugar of different cultivars of okra.

4.3.4 Fiber

The dietary fiber plays an important role in decreasing the risk of many disorders such as constipation, diabetes, cardiovascular diseases and obesity (Nair *et al.*, 2013).

Fiber content was observed higher inGAO-5 (1.067 mg/g), which was statistically at par with cultivar GO-2 (1.0 mg/g), Nirmala-303 (1.0 mg/g) and Parbhani kranti(0.767 mg/g), while lower fiber content was observed in cultivar Glory (0.433 mg/g) and Arka Anamika (0.433 mg/g) followed by Ganesh (0.467 mg/g), Akur-40 (0.467 mg/g) and Shive (0.633 mg/g) (Table 4.3).

Patel *et al.* (2012) studied 10 different cultivars of okra in which higher fiber content was found in AOL-05-1(2.64%) and lower fiber content was found in Parbhani kranti (0.49%).

4.3.5 Total soluble sugars

Sugars plays an important role in osmotic adjustment and the main role of soluble sugars is the protection against various types of stresses.

The highest total soluble sugars content was observed in Glory (2.9 %), which was statistically at par with Arka Anamika (2.84%), Ganesh (2.77%), Jyoti (2.73%) and Shive (2.72%). The lest total soluble sugars content was recorded for GAO-5 (2.31 %), which was at par with Parbhani kranti (2.57%) and GO-2 (2.5%) (Table 4.3, & Fig.4.3).

Ruby *et al.* (1993) reported that total soluble sugar ranged between 2.28 – 5.22 g/100g in okra fruit.

4.3.6 Reducing sugars

Sugars are the primary products of photosynthesis and perform multiple roles in plants as energy and carbon transport molecules, hormone-like signalling factors, from which plants make proteins, polysaccharides, oils and woody materials

The highest reducing sugars content was recorded (Table 4.3, & Fig.4.3) in Glory and Arka Anamika (0.55%) followed by Ganesh (0.53%), Jyoti (0.52%), Shive (0.52%) and Ankur (0.49%). The least reducing sugars content was recorded in GAO-5 (0.44%) cultivar, which was statistically at par with Parbhani kranti (0.50%), GO-2 (0.50%) and Nirmala-303 (0.51%).

Rahman *et al.* (2011) studied the effect of fertilizer on okra with different treatments in which they found that the highest reducing sugar content was with T_2 treatment (N_0P_1K1, 2 kg cow dung, 65 g urea, 150 g TSP, and 105 g PM) (1.11 %).

4.3.7 Non reducing sugars

The highest non reducing sugars content was found (Table 4.3, & Fig.4.3) in Glory (2.36%) followed by Arka Anamika (2.31%), Ganesh (2.28%), Jyoti (2.11%) and Shive (2.21), and the least non reducing sugar content was recorded in GAO-5 (1.81%), which was significantly at par with Parbhani kranti and GO-2 (2.03 %).

4.3.8 Total phenols

Phenolic compounds are plant secondary metabolites those constitute one of the most common and widespread groups of substances in plants. Plants need phenolic compounds for pigmentation, growth, reproduction, resistance to pathogens and for many other functions. Genotype GAO-5 had the highest phenol content 0.086 %, while the cultivar Ganesh recorded the least phenol content 0.055 %, which was statistically at par with Arka Anamika (0.059 %) and Ganesh (0.064%) (Table 4.4, & Fig.4.4).

Kabir and Pillu (2011) observed that phenol content in okra fruit of Arka Anamika (167.62mg/100g) was significantly higher than Sinnova (112.27mg/100g) and Shagun (106.26mg/100g).

Table 4.4:- Phenol, true protein, antioxidant activity and ascorbic acid of different cultivars from okra fruits

Cultivar	Phenol (%)	True protein (%)	Antioxidant activity (%)	Ascorbic acid (mg/100g)
Ankur-40	0.069	1.64	0.41	33.58
Arka Anamika	0.059	1.94	0.36	32.69
GAO -5	0.086	1.78	0.49	39.66
GO – 2	0.083	1.73	0.47	39.04
Ganesh	0.064	1.92	0.38	34.57
Glory	0.055	1.92	0.40	36.44
Jyoti	0.075	1.85	0.47	37.12
Nirmala-303	0.082	1.97	0.44	39.66
Parbhani Kranti	0.082	1.86	0.40	37.12
Shive	0.067	1.92	0.35	39.04
S.Em.±	0.003	0.04	0.03	2.68
C.D.@5%	0.009	0.11	0.08	7.96
CV%	7.645	3.41	10.97	12.58

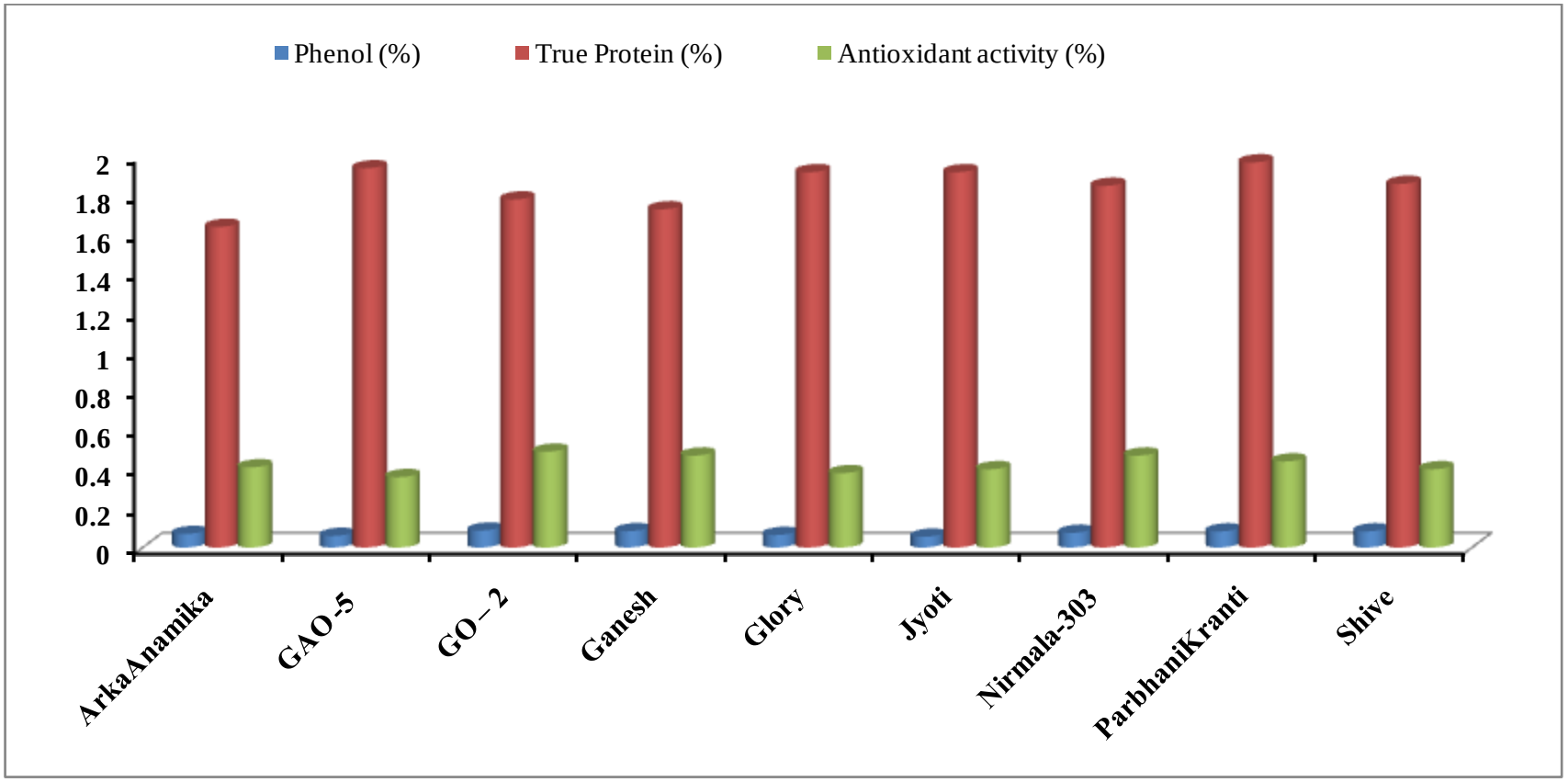


Fig. 4.4:- Phenol, true protein and antioxidant activity of different cultivars of okra

4.3.9 True protein

Protein is an important biochemical factor for disease resistance in plant. The maximum protein content was observed in Nirmala-303 (1.97%) followed by Arka Anamika (1.94%), while the minimum protein content was observed in Ankur-40 (1.64%) followed by GO-2 (1.73 %) and GAO-5(1.78%) (Table 4.4, & Fig.4.4).

Nair *et al.* (2013) reported 0.948 mg/g protein in fruit of Arka Anamika cultivar.

2.4.10 Total Antioxidant activity

Antioxidants neutralize the effect of free radicals through different ways and may prevent the body from various diseases.

The maximum and minimum total antioxidant activity was recorded for GAO-5 (0.49%) and Shive (0.35%), respectively (Table 4.4, & Fig.4.4). Though higher antioxidant activity was found in GAO-5 (0.49%) but it was statistically at par with GO-2 (0.47%), Jyoti (0.47%), and Nirmala-303 (0.44%).

Khomsug *et al.* (2010) observed that the pulp of okra had high relative antioxidant activity about 44.1 mg/ml.

4.3.11 Ascorbic acid

Ascorbic acid is an abundant component of plant. It functions in photosynthesis as an enzyme co-factor and in control of cell growth. It also functions as an antioxidant, an enzyme co-factor, resistance to environmental stresses and synthesis of ethylene, gibberellins, anthocyanins and hydroxyproline.

Ascorbic acid content was observed the highest in cultivar GAO-5 and Nirmala-303, (39.66 mg/100g) followed by GO-2 (39.04 mg/100g), while lower ascorbic acid content was observed in cultivar Gory (36.44 mg/100g) (Table 4.4).

Kabir and Pillu (2011) studied ascorbic acid content of different cultivars it initially increased with maturity but later on with advancement of maturity it reduced significantly. Ascorbic acid content was was the least in Shagun (12.31mg/100g) followed by Arka Anamika (10.82mg/100g) and Sinnova (10.18mg/100g).

4.3.12 Total chlorophyll

Chlorophyll is a light-absorbing pigment, and it actually gets its green color because it absorbs blue and red wavelengths of light. Since it is a light absorbing pigment, chlorophyll is also called a photoreceptor.

Total chlorophyll was the maximum in cultivar GAO-5 1.51 mg/g, which was statistically at par with GO-2 (1.45 mg/g), Nirmala-303(1.44 mg/g) and Parbhani kranti (1.43 mg/g), while the minimum chlorophyll content was recorded in Glory 1.17 mg/g (Table 4.3, & Fig.4.3).

Patel *et al.* (2012) studied ten different cultivars of okra among which the highest chlorophyll content was found in AOL-8-5 (0.56 mg/g) and the least chlorophyll content was found in AOL-03-1 (0.18 mg/g).

Table 4.5:- Chlorophyll a, chlorophyll b, chlorophyll a/b, total chlorophyll and total carotenoid of different cultivars from okra fruits

Cultivar	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll A/B (mg/g)	Total chlorophyll (mg/g)	Total carotenoids (mg/g)
Ankur-40	0.87	0.38	2.35	1.25	0.056
Arka Anamika	0.85	0.39	2.25	1.23	0.028
GAO -5	0.98	0.53	1.85	1.51	0.084
GO – 2	0.91	0.55	1.67	1.45	0.085
Ganesh	0.88	0.36	2.50	1.24	0.047
Glory	0.91	0.26	3.47	1.17	0.038
Jyoti	0.91	0.36	2.62	1.26	0.064
Nirmala-303	0.90	0.54	1.68	1.44	0.068
Parbhani Kranti	0.90	0.53	1.70	1.43	0.076
Shive	0.89	0.44	2.14	1.33	0.067
S.Em.±	0.03	0.04	0.22	0.03	0.004
C.D.@5%	0.09	0.10	0.67	0.10	0.011
CV%	5.61	14.04	17.51	4.18	10.72

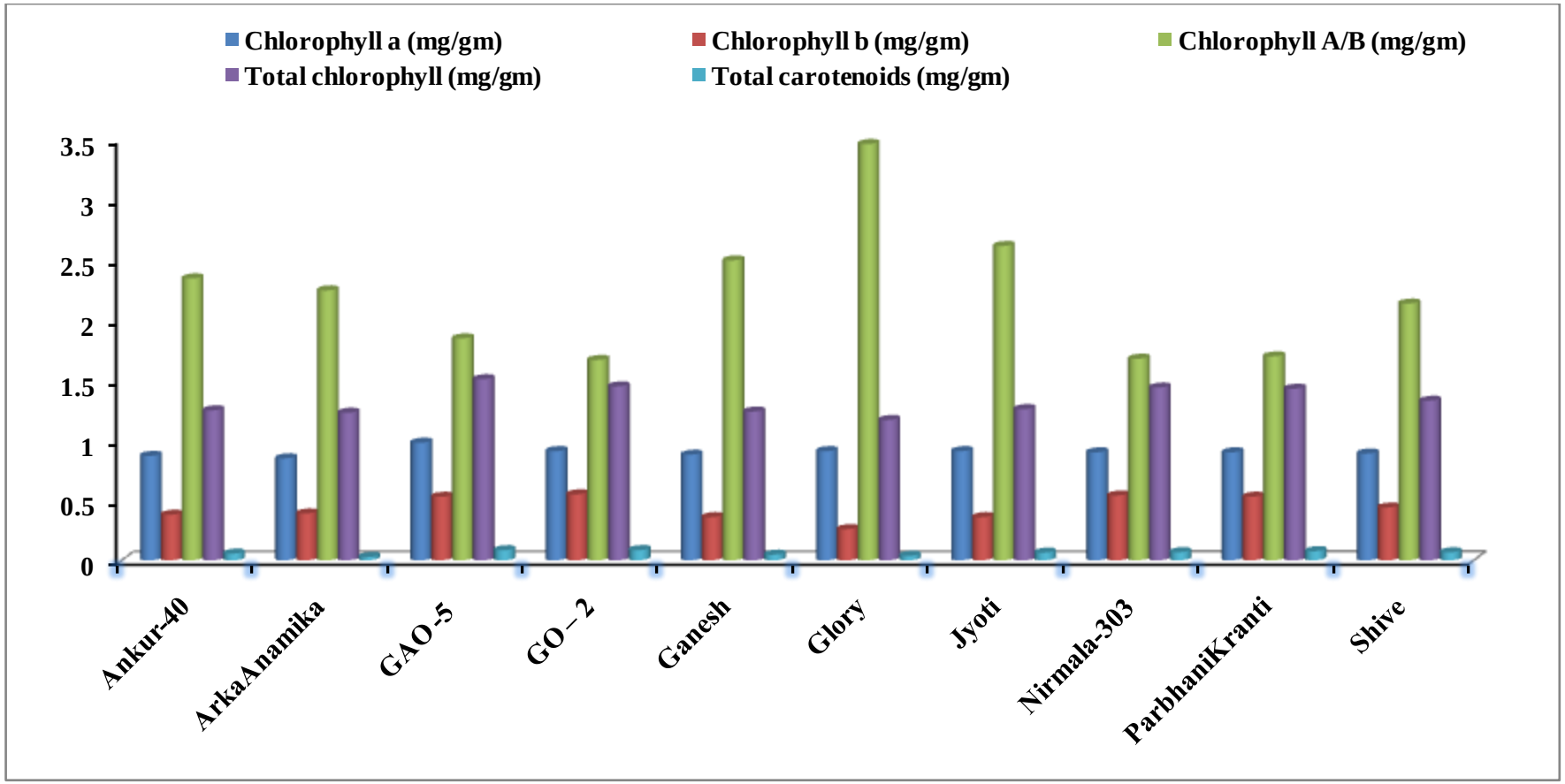


Fig. 4.5:- Chlorophyll a, chlorophyll b, chlorophyll A/B and total chlorophyll of different cultivars of okra

4.3.13 Chlorophyll a

Chlorophyll a content was significant among okra cultivars (Table 4.5, & Fig.4.5).The maximum and minimum chlorophyll a was found in GAO-5 (0.98 mg/g) and Arka Anamika (0.85 mg/g), respectively.

4.3.14 Chlorophyll b

Chlorophyll b content was also significant among all okra cultivars (Table 4.3, & Fig.4.3). The maximum chlorophyll b was recorded with GO-2 (0.55 mg/g), which was followed by Nirmala-303 (0.54 mg/g), GAO-5 (0.53 mg/g) and Parbhani kranti (0.53 mg/g), where as, minimum chlorophyll b was observed in Glory (0.26 mg/g), which was statistically at par with Ganesh and Jyoti (0.36 mg/g). (Table 4.5, & Fig.4.5).

4.3.15 Chlorophyll a/b

Chlorophyll a/b ratio ranged from 1.67 to 3.47 (Table 4.5, & Fig.4.5). Glory had the highest chlorophyll a/b ratio (3.47), while culivar GO-2 (1.67) was recorded the lowest ratio.

4.3.16 Total carotenoids

Carotene plays a crucial role as a photosynthetic pigment there by important for photosynthesis. It does not actively contribute in photosynthesis, but it transmits the energy, it absorbs by chlorophyll, and also plays a protective role as chlorophyll being a powerful antioxidant that protects organic molecules from being destroyed by oxidation.

Total carotenoids content was observed the highest in genotype GO-2 0.085 mg/g, which was statistically at par with GAO-5(0.084 mg/g) and Parbhani kranti (0.076 mg/g), while the least carotenoids content was observed in cultivar Arka

Anamika 0.028 mg/g, which was statistically at par with genotype Glory (0.038 mg/g) (Table 4.5, & Fig.4.5).

Nair *et al.* (2013) observed the okra fruit as contained cultivar Arka Anamika 0.624 mg/g.

4.4 Enzyme Activity

4.4.1 Peroxidase activity (Δ O.D. min^{-1} g^{-1}fw)

Maximum peroxidase activity was recorded in genotype GAO-5 (17.84 Δ O.D. min^{-1} g^{-1}fw), which was statistically at par with Nirmala-303. Minimum peroxidase activity was recorded in cultivar Glory (14.59Δ O.D. min^{-1} g^{-1}fw), which was significantly at par with Ganesh (Table 4.6, & Fig.4.6).

Peroxidase does not play direct role in resistance against pathogen but plant disease resistance has often been correlated with elevated peroxidase activity and the oxidation of phenolics in diseased tissues. The role of peroxidase in plant defense mechanism has been attributed to its ability to oxidize key metabolites in plant or pathogen (Chittor *et al., 1999*). Peroxidase is also involved in lignin synthesis and degradation of cytotoxic levels of hydrogen peroxide enerated in plant tissues as a result of pathogen attack (Van loon, 1997).

4.4.2 Polyphenol oxidase activity (Δ O.D. min^{-1} g^{-1}fw)

Maximum polyphenol oxidase activity was recorded in genotype GAO-5 (11.40Δ O.D. min^{-1} g^{-1}fw), which was significantly at par with Nirmala-303. Minimum polyphenol oxidase activity was recorded in genotype Glory (9.56Δ O.D. min^{-1} g^{-1}fw), (Table 4.6, & Fig.4.6). Polyphenol oxidase seems to be compartmentalized in plant cells but as a result of pathogenic attack or during tissue senescence, membrane disruption may occur which can initiate formation of quinones

following an increase in accessibility of PPO to its substrate (Mohammadi and Kazemi, 2002).

4.4.3 Catalase activity (Δ O.D. min^{-1} g^{-1}fw)

Maximum catalase activity was recorded in genotype GAO-5 (0.627Δ O.D. min^{-1} g^{-1}fw), which was statistically at par with GO-2 (0.564Δ O.D. min^{-1} g^{-1}fw), Ganesh (0.502Δ O.D. min^{-1} g^{-1}fw) and Nirmala-303 (0.500Δ O.D. min^{-1} g^{-1}fw), while minimum catalase activity was recorded in cv. Glory(0.139Δ O.D. min^{-1} g^{-1}fw), (Table 4.6, & Fig.4.6).

Table 4.6:- Peroxidase, polyphenol oxidase and catalase activity of different cultivars of okra.

Cultivar	Peroxidase (Δ O.D. min^{-1} g^{-1}fw)	Polyphenol oxidase (Δ O.D. min^{-1} g^{-1}fw)	Catalase (Δ O.D. min^{-1} g^{-1}fw)
Ankur-40	15.51	10.139	0.289
Arka Anamika	15.07	9.997	0.398
GAO -5	17.84	11.398	0.627
GO – 2	16.67	11.071	0.564
Ganesh	14.77	9.848	0.502
Glory	14.59	9.56	0.139
Jyoti	16.97	11.014	0.459
Nirmala-303	17.03	11.277	0.500
Parbhani Kranti	15.78	10.324	0.380
Shive	16.51	10.50	0.268
S.Em.I	0.17	0.13	0.07
C.D.@5%	0.51	0.39	0.21
CV%	1.84	2.15	30.05

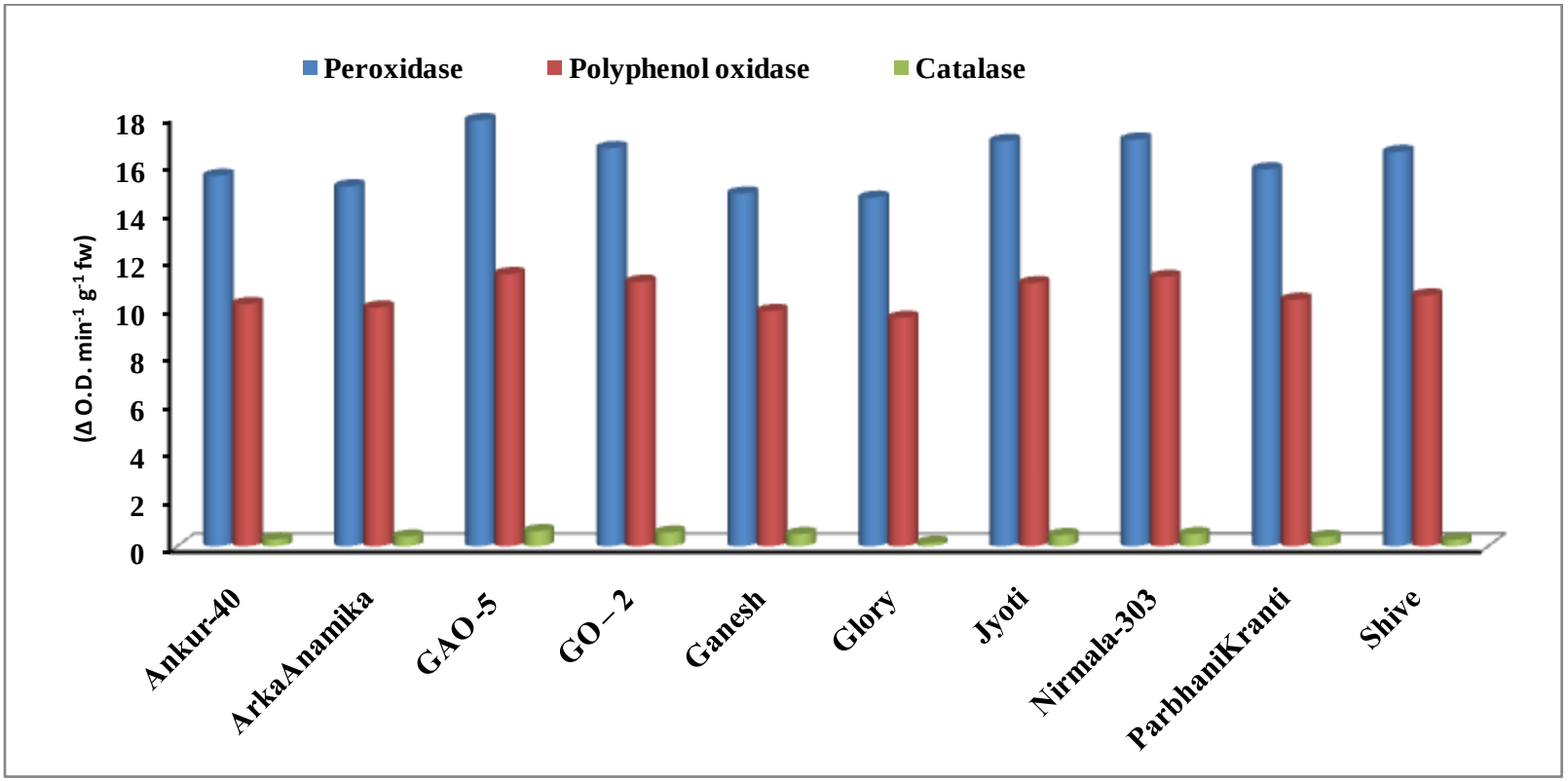


Fig4.6: Peroxidase, polyphenol oxidase and catalase activity of different okra cultivars.

4.5 SDS-PAGE of okra fruit protein

The total proteins were fractioned into 12 bands, which stated heterogeneity among different cultivars (Plate 4.1). The maximum number of bands (11) were observed in GAO-5 followed by GO-2 (10), Shive (10), Jyoti (9) and Ankur-40 (9), while minimum number of bands 5 were observed in cultivar Glory. The cultivar GAO-5 and GAO-2 were differentiated by one unique band having Rm value 0.491 (Table 4.7). 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.

Table 4.7: Banding pattern of total protein

Cultivars		Ankur-40	Arka Anamika	GAO-5	GO-2	Ganesh	Glory	Jyoti	Nirmala-303	Parbhani Kranti	Shive
Moleculr weight kD	Rm values										
392.41	0.087	+	+	+	+	+	+	+	+	+	+
352.73	0.105	+	+	+	+	+	+	+	+	+	+
183.26	0.213	+	+	+	+	+	+	+	+	+	+
137.22	0.261	+	+	+	+	+	-	+	+	+	+
96.67	0.319	+	-	-	+	-	-	+	-	-	-
58.48	0.402	-	-	+	-	+	-	+	-	-	+
34.52	0.491	-	-	+	+	-	-	-	-	-	-
28.16	0.523	+	-	+	+	+	-	+	-	-	+
20.76	0.574	+	-	+	+	+	-	-	-	-	+
15.31	0.624	-	+	+	+	+	+	+	+	+	+
9.85	0.697	+	+	+	+	+	+	+	+	+	+
6.05	0.778	+	+	+	-	+	-	-	-	-	+

Jaccard's similarity coefficient on the basis of presence and absence of bands was calculated for all possible pairs of 10 cultivars of okra (Table 4.8). The highest similarity

index value was 1.00, while the least similarity index value was 0.40. The average similarity coefficient among cultivars was 0.78 (Table 4.8). Based on this similarity index a dendogram was prepared and shown in Fig 4.7.

4.5.1 Clustering pattern of Total protein

Two clusters *viz.* A and B were formed at a similarity coefficient of 0.57 (Fig. 4.8). Sub-cluster of A_1 included one cluster in which consisted of two cultivars *viz.*, Ankur-40 andGO-2. Sub-cluster A_2consistedtwo minor clusters in which one consisted of three cultivars *viz.*, GAO-5, Ganesh and Shive another minor cluster consisted of only one cultivar Jyoti. The cluster B represented cultivars which were divided in to two sub-clusters B_1 and B_2. Sub-cluster B_1 consisted of cultivars Arka Anamika, Nirmala-303 and Parbhani kranti. Sub-cluster B_2 included alone cv. Glory.

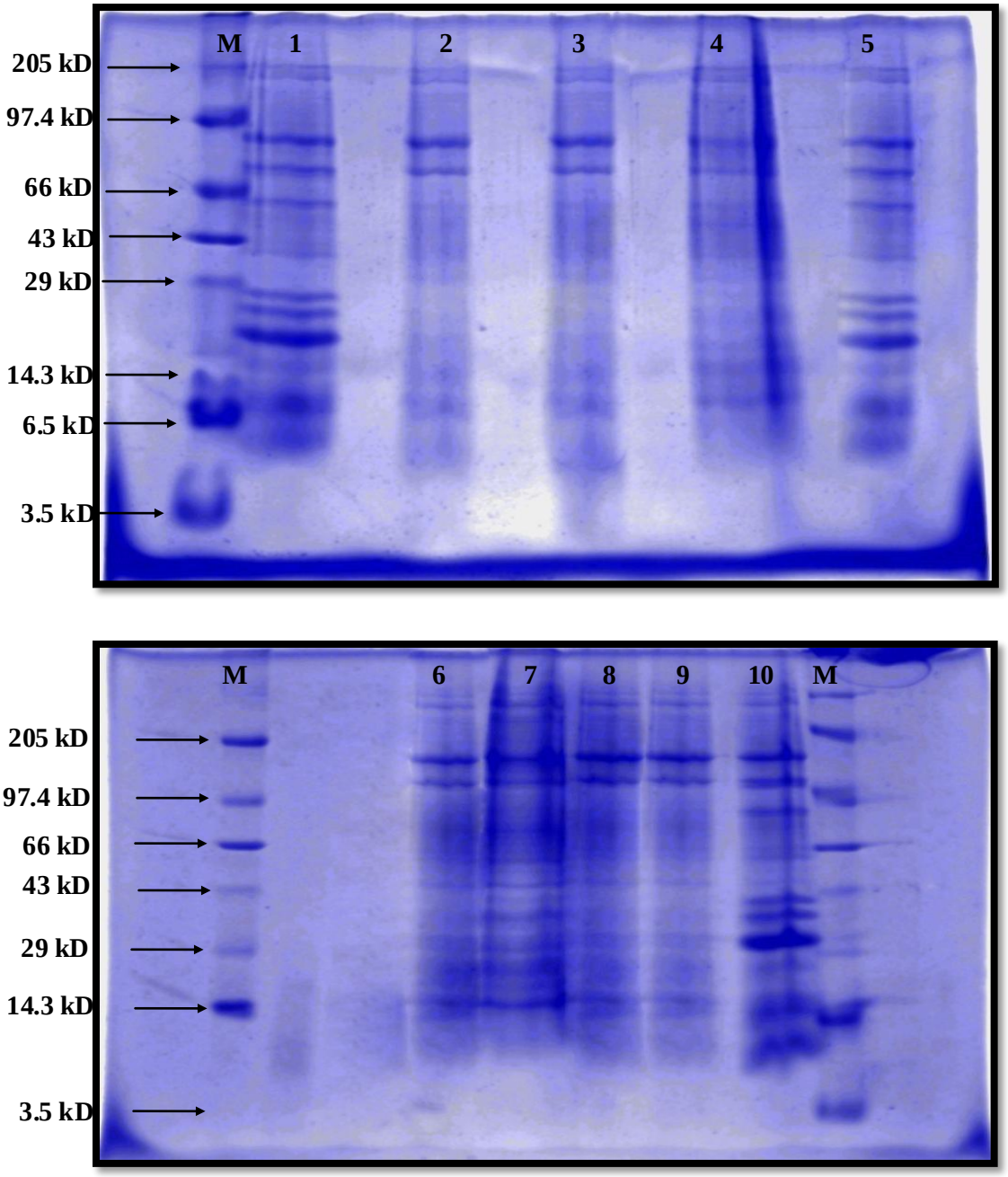


Plate 4.1: SDS PAGE of fruit protein from different okra cultivars

M = Protein marker (1) Ankur-40 (2) Arka Anamika (3) GAO -5 (4) GO-2 (5) Ganesh (6) Glory (7) Jyoti (8) Nirmala-303 (9) Parbhani kranti (10) Shive

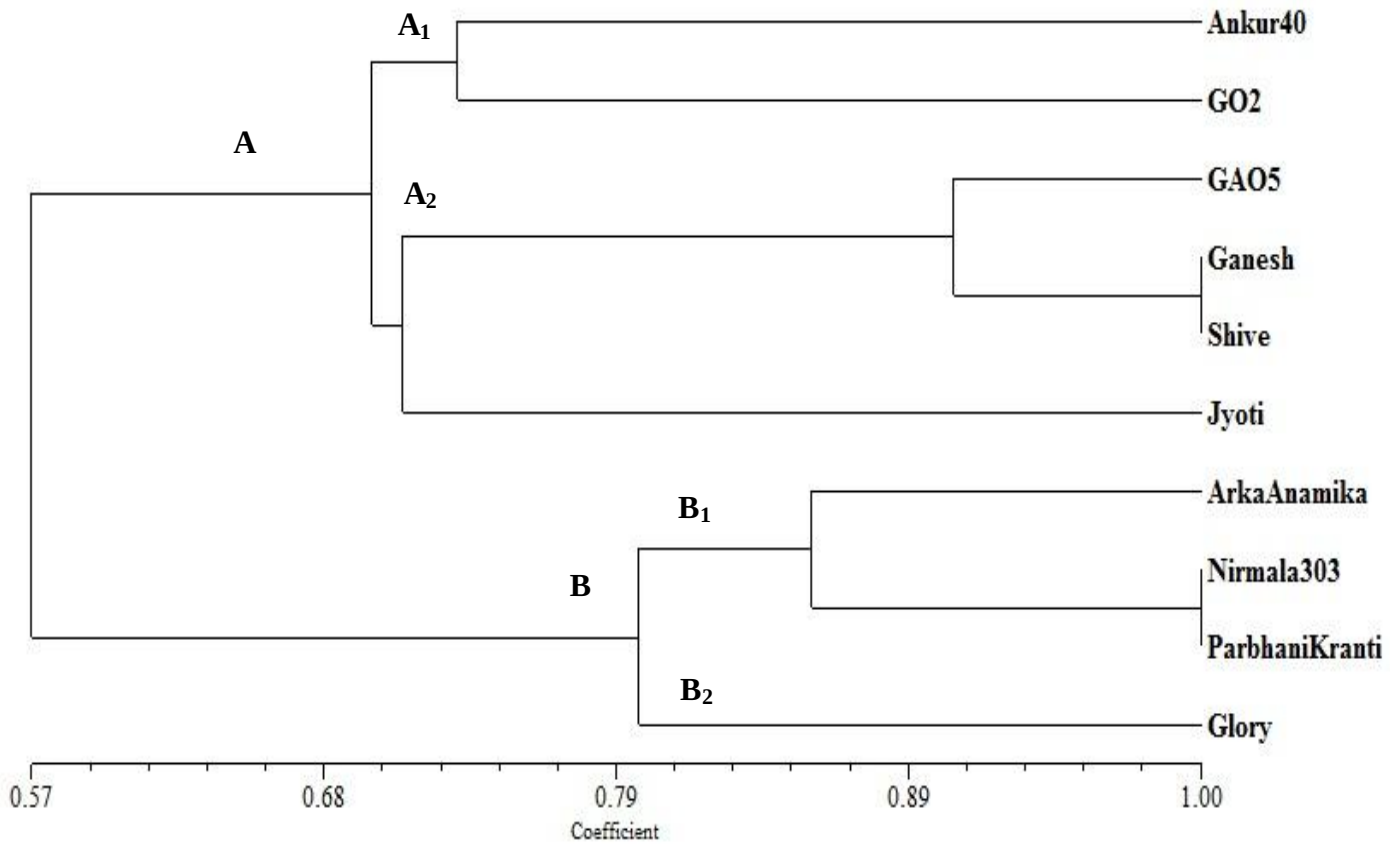


Fig. 4.7: Dendogram showing clustering of ten okra cultivars constructed obtained from total protein analysis

Table 4.8: Genetic similarity matrix of total protein data based on Jaccard's similarity coefficient

	Ankur-40	Arka Anamika	GAO-5	GO-2	Ganesh	Glory	Jyoti	Nirmala-303	Parbhani Kranti	Shive
Ankur-40	1.00									
Arka Anamika	0.60	1.00								
GAO-5	0.67	0.64	1.00							
GO-2	0.73	0.55	0.75	1.00						
Ganesh	0.73	0.70	0.91	0.67	1.00					
Glory	0.40	0.71	0.45	0.50	0.50	1.00				
Jyoti	0.64	0.60	0.67	0.73	0.73	0.56	1.00			
Nirmala-303	0.50	0.86	0.55	0.60	0.60	0.83	0.67	1.00		
Parbhani Kranti	0.50	0.86	0.55	0.60	0.60	0.83	0.67	1.00	1.00	
Shive	0.73	0.70	0.91	0.67	1.00	0.50	0.73	0.60	0.60	1.00

4.6 Isozyme study

4.6.1 Peroxidase isozyme

The isozyme pattern of peroxidase was observed among different okra cultivars (Plate 4.2). Total 6 types of bands were observed. The maximum numbers of bands 6 were observed in GAO-5. Band no. 1 and 5 were not observed for Ankur-40 and Arka Anamika, while the band no. 5 was absent in Jyoti, Nirmala-303, Parbhani kranti and Shive and band no. 4 was absent in GO-2, Ganesh and Glory. The band having Rm value 0.335 and 0.497 were observed in all cultivars (Table 4.9). Lagrimini and Rothstein (1987) observed specific peroxidase band (ph 6.1) in TMV infected tobacco leaves.

Table 4.9: Banding pattern of peroxidase isozyme

Culivars	Ankur -40	Arka Anamika	GAO -5	GO -2	Ganesh	Glory	Jyoti	Nirmala -303	Parbhani Kranti	Shive
Rm values										
0.135	-	-	+	+	+	+	+	+	+	+
0.292	+	+	+	+	+	+	+	-	-	-
0.335	+	+	+	+	+	+	+	+	+	+
0.411	+	+	+	-	-	-	+	+	+	+
0.449	-	-	+	+	+	+	-	-	-	-
0.497	+	+	+	+	+	+	+	+	+	+

Jaccard's similarity coefficient was calculated for all possible pairs of 10 cultivars of okra (Table 4.10). The highest and the lowest similarity index values were found 1 and 0.5, respectively. Based on this similarity index a dendogram was prepared and shown in Fig 4.8(a). Here one cluster contained Ankur-40, Arka Anamika, Jyoti, Nirmala-303, Parbhani kranti and Shive, where as, other cluster contained two sub clusters one for GAO-5 and one for GO-2, Ganesh and Shive cultivars.

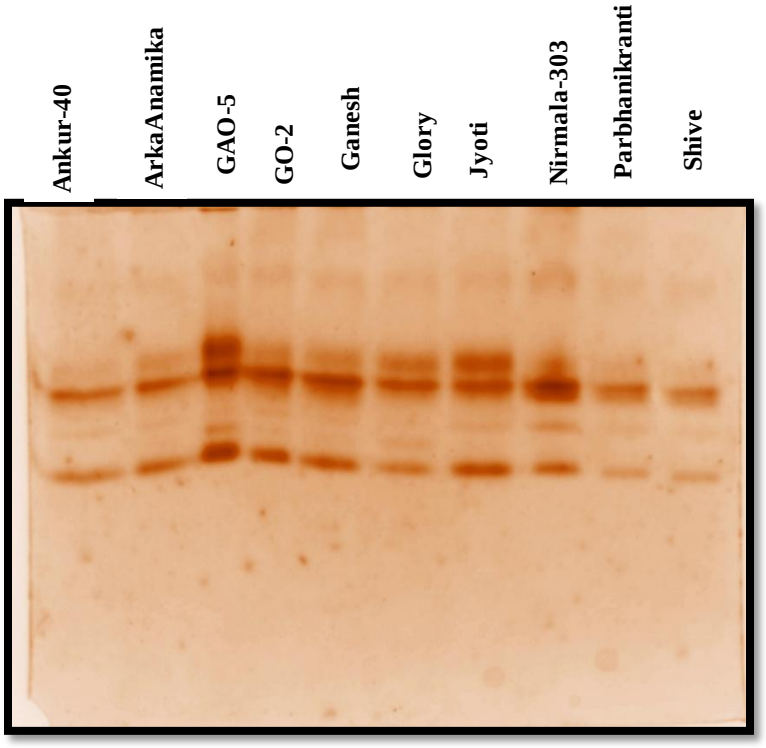


Plate 4.2: Peroxidase banding pattern from different okra cultivars

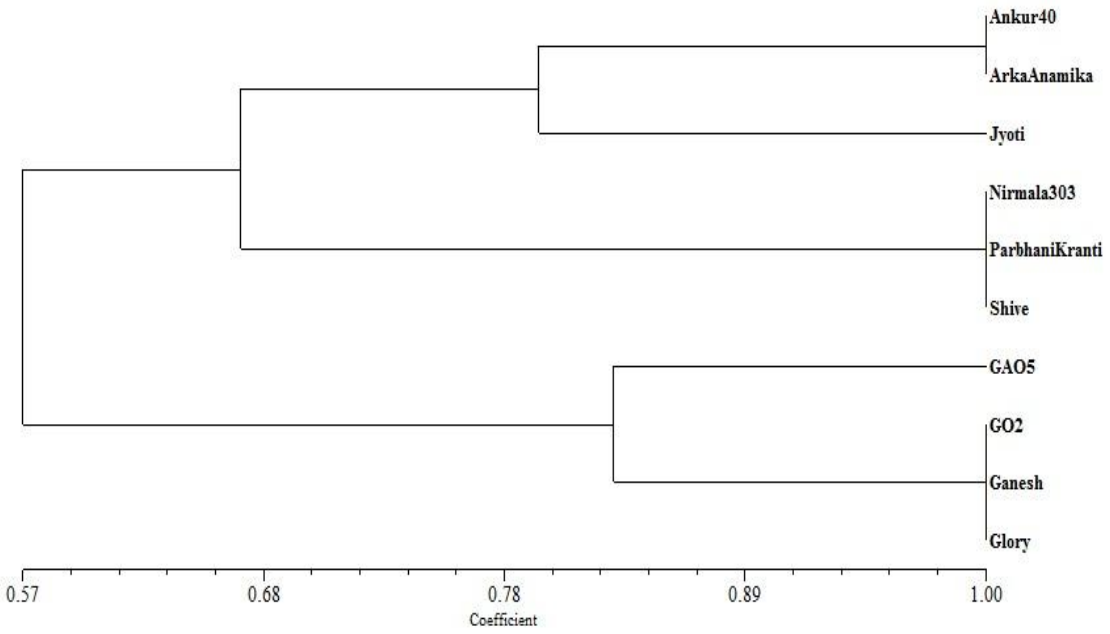


Fig. 4.8(a): Dendogram showing clustering of peroxidase isozyme

Table 4.10: Genetic similarity matrix of peroxidase data based on Jaccard's similarity coefficient

	Ankur-40	Arka Anamika	GAO-5	GO-2	Ganesh	Glory	Jyoti	Nirmala-303	Parbhani Kranti	Shive
Ankur-40	1									
Arka Anamika	1	1								
GAO-5	0.67	0.67	1							
GO-2	0.5	0.5	0.83	1						
Ganesh	0.5	0.5	0.83	1	1					
Glory	0.5	0.5	0.83	1	1	1				
Jyoti	0.8	0.8	0.83	0.67	0.67	0.67	1			
Nirmala-303	0.6	0.6	0.67	0.5	0.5	0.5	0.8	1		
Parbhani Kranti	0.6	0.6	0.67	0.5	0.5	0.5	0.8	1	1	
Shive	0.6	0.6	0.67	0.5	0.5	0.5	0.8	1	1	1

4.6.2 Polyphenol oxidase isozyme

The PPO isozyme was observed among different okra cultivars (Plate 4.3). PPO was observed with total three different types of bands. Arka Anamika, GAO-5 and Jyoti cultivars depicted with all three bands. The band having Rm value 0.225 was observed in all cultivars. Band no. 1 and 3 were absent in Ankur-40, Glory, Nirmala-303 and Parbahni kranti. The cultivars Arka Anamika, GAO-5 and Jyoti were differentiated by one unique bands having Rm value 0.344 (Table 4.11). Similar type of study was carried out by Khorsheduzzaman *et al.* (2010) in different brinjal cultivars.

Table 4.11: Banding pattern of PPO isozyme

Cultivars	Ankur-40	Arka Anamika	GAO-5	GO-2	Ganesh	Glory	Jyoti	Nirmala-303	Parbhani Kranti	Shive
Rm values										
0.120	-	+	+	+	+	-	+	-	-	+
0.225	+	+	+	+	+	+	+	+	+	+
0.344	-	+	+	-	-	-	+	-	-	-

Jaccard's similarity coefficient was calculated for all possible pairs of ten cultivars of okra (Table 4.12). The lowest similarity index value was 0.33, whereas the highest similarity index value was 1.0.Based on this similarity index a dendogram was prepared and shown in Fig 4.8(b).

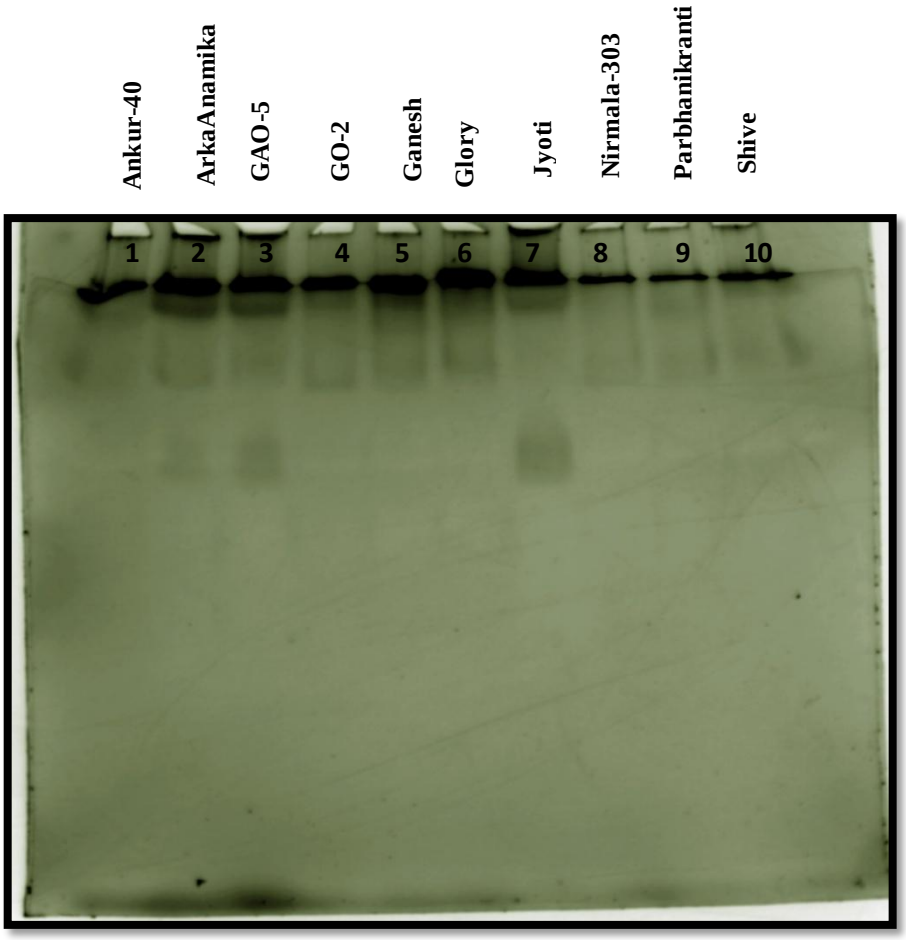


Plate 4.3: PPO banding pattern from different okra genotypes

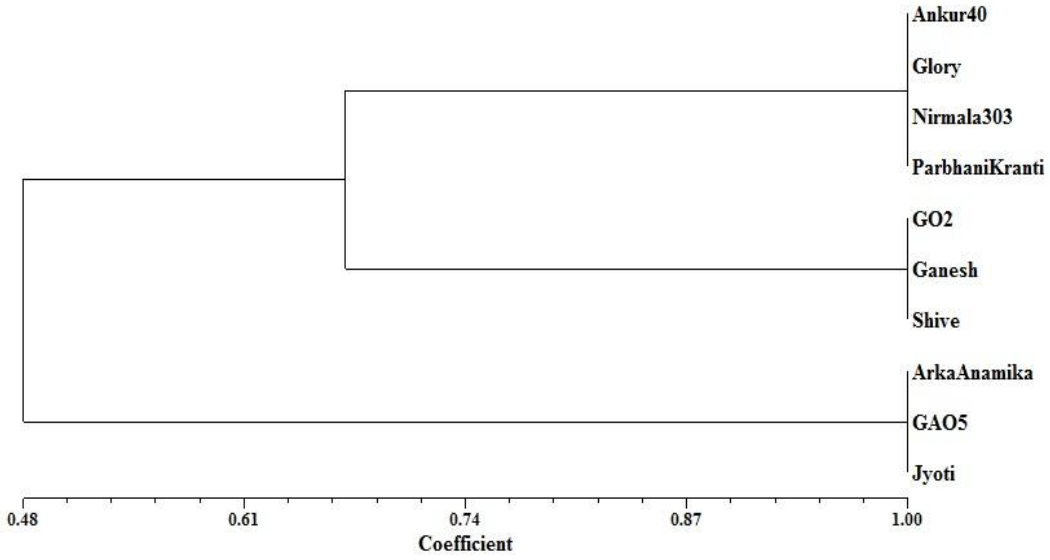


Fig. 4.8(b): Dendogram showing clustering of PPO isozyme

Table 4.12: Genetic similarity matrix of PPO data based on Jaccard's similarity coefficient

	Ankur-40	Arka Anamika	GAO-5	GO-2	Ganesh	Glory	Jyoti	Nirmala-303	Parbhani Kranti	Shive
Ankur-40	1.00									
Arka Anamika	0.33	1.00								
GAO-5	0.33	1.00	1.00							
GO-2	0.67	0.67	0.67	1.00						
Ganesh	0.67	0.67	0.67	1.00	1.00					
Glory	1.00	0.33	0.33	0.67	0.67	1.00				
Jyoti	0.33	1.00	1.00	0.67	0.67	0.33	1.00			
Nirmala-303	1.00	0.33	0.33	0.67	0.67	1.00	0.33	1.00		
Parbhani Kranti	1.00	0.33	0.33	0.67	0.67	1.00	0.33	1.00	1.00	
Shive	0.67	0.67	0.67	1.00	1.00	0.67	0.67	0.67	0.67	1.00

4.6.3 Super oxide dismutase

The SOD isozyme was observed among different okra cultivars (Plate 4.4). SOD was observed with total 6 different types of bands. Ankur-40, Arka Anamika, and Glory cultivars depicted all six bands. The band having Rm value 0.137 was observed in all cultivars. The cultivars Ankur-40, Arka Anamika and Glory were differentiated by one unique band having Rm value 0.544 (Table 4.13).

Table 4.13: Banding pattern of SOD isozyme

Cultivars	Ankur-40	Arka Anamika	GAO-5	GO-2	Ganesh	Glory	Jyoti	Nirmala-303	Parbhani Kranti	Shive
Rm values										
0.085	++	+	-	+	-	+	-	-	+	+
0.137	++	+	+	+	+	+	+	+	+	+
0.392	+	+	-	+	-	+	-	-	-	-
0.454	+	+	+	-	+	+	+	+	+	+
0.471	++	+	-	+	+	+	+	+	+	+
0.544	+	+	-	-	-	+	-	-	-	-

Jaccard's similarity coefficient was calculated for all possible pairs of 10 cultivars of okra (Table 4.14). The highest and the lowest similarity index values were found 1 and 0.33, respectively. Based on this similarity index a dendogram was prepared and shown in Fig 4.8(c). Here one cluster contained Ankur-40, Arka Anamika, Glory, GO-2 and Shive where as other cluster contained two sub clusters, one for GAO-5 and one for Ganesh, Jyoti,Nirmala-303 and Parbhani kranti cultivars.

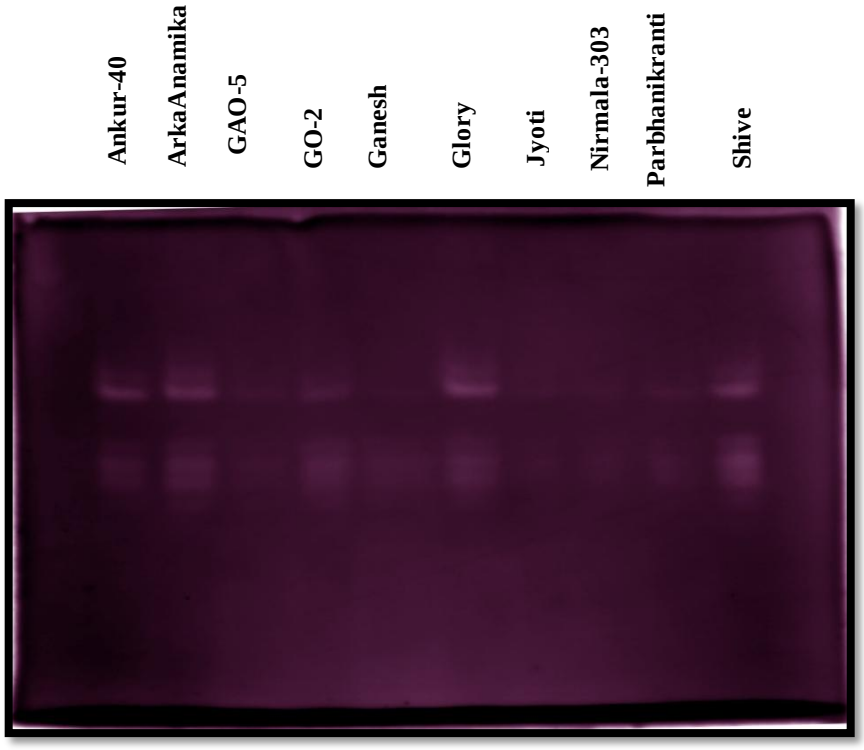


Plate 4.4: SOD banding pattern from different okra cultivar

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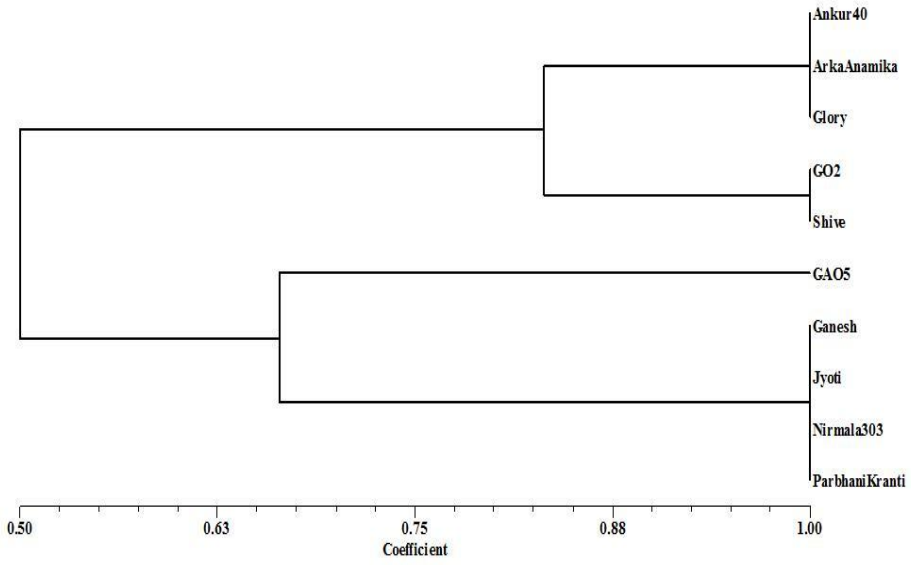

Fig. 4.8(c): Dendogram showing clustering of SOD isozyme

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

	Ankur-40	Arka Anamika	GAO-5	GO-2	Ganesh	Glory	Jyoti	Nirmala-303	Parbhani Kranti	Shive
Ankur-40	1									
Arka Anamika	1	1								
GAO-5	0.33	0.33	1							
GO-2	0.83	0.83	0.4	1						
Ganesh	0.5	0.5	0.67	0.6	1					
Glory	1	1	0.33	0.83	0.5	1				
Jyoti	0.5	0.5	0.67	0.6	1	0.5	1			
Nirmala-303	0.5	0.5	0.67	0.6	1	0.5	1	1		
Parbhani Kranti	0.5	0.5	0.67	0.6	1	0.5	1	1	1	
Shive	0.83	0.83	0.4	1	0.6	0.83	0.6	0.6	0.6	1

4.7 SSR markers analysis

In present investigation, 10 okra cultivars were subjected to SSR analysis using different primers of OK series. From those 8 primers *viz.* as OK-1, OK-2, OK-3, OK-4, OK-6, OK-7, OK-9 and OK-12 were amplified. As mentioned in section 3.8.3, various bio-statistical parameters were calculated, which are efficient for diversity analysis. The SSR primers have been designed from the data against YVMV disease carried out at the Anand Agricultural University, Anand. (Patel J. S., 2013).

Table 4.15: Results of SSR analysis

Sr. No	Locus name	No. of bands amplified	Molecular size range (bp)	Total no of alleles	PIC	Na	Ne	He
1	OK-1	17	251-292bp	2	0.48	2	1.98	0.495
2	OK-2	16	284-557bp	7	0.81	7	1.10	0.097
3	OK-3	10	283-360bp	1	0	1	2.27	0.00
4	OK-4	9	273bp	1	0	1	1.00	0.432
5	OK-6	10	258-271bp	4	0.7	4	1.76	0.189
6	OK-7	10	214bp	1	0	1	1.00	0.000
7	OK-9	9	331bp	2	0.19	2	1.10	0.097
8	OK-12	9	143bp	1	0	1	1.76	0.432
Total		**90**	**-**	**19**	**2.19**	**19**	**11.97**	**2.081**
Average		**11.25**	**143-557bp**	**2.37**	**0.27**	**2.37**	**1.49**	**0.26**

Na = No. of different Alleles**,**

Ne = No. of effective Alleles,

He = Expected heterozygosity

Table 4.16: Allele description of SSR analysis

Sr. No	Locus name	Allele designation	Allele length (bp)	Allele frequency
1	OK-1	A	251	0.45
		B	292	0.55
2	OK-2	A	261	0.12
		B	266	0.15
		C	268	0.10
		D	470	0.20
		E	496	0.15
		F	500	0.15
		G	557	0.13
3	OK-3	A	360	1.00
4	OK-4	A	273	1.00
5	OK-6	A	270	0.20
		B	267	0.10
		C	264	0.30
		D	262	0.40
6	OK-7	A	214	1.00
7	OK-9	A	331	0.32
		B	330	0.68
8	OK-12	A	143	1.00

4.7.1 Polymorphism pattern of SSR

The data obtained in the present investigation are presented in Table 4.15 and Table 4.16. In the present SSR analysis, 19 alleles were produced by 8 amplified markers. The average number of alleles per locus was found to be 2.37, while effective number of alleles was found 1.49. The maximum number of alleles (7) were recorded for markers OK-2, while marker OK-6 produced 4 alleles. The highest allele frequency found by marker OK-9, and it was 0.68. The highest PIC value (0.81) was recorded for OK-2. The average PIC value and number of effective alleles were 0.27

and 1.49 respectively. The molecular weight of the amplified PCR products ranged from 143 bp (OK-11) to 557 bp (OK-2) (Table 4.15).

Based on the SSR data, cluster analysis was performed using genetic similarity values and a dendogram was generated showing the grouping of cultivars. The highest similarity index value 0.82 was found between Jyoti and Nirmala-303, whereas, the least similarity index value 0.23 was found between GAO-5 and Parbhani kranti. (Table 4.17).

4.7.2 Clustering pattern of different SSR primers used

Clustering pattern of dendogram was generated by using the pooled molecular data of 8 SSR loci indicated that two clusters *viz.* A and B were formed at a similarity coefficient of 0.44 (Fig. 4.9). Sub-cluster of A_1 included two minor clusters in which one consisted of two cultivar *viz.* Ankur-40 and Arka Anamika another minor cluster included a cultivar Glory. Sub-cluster of A_2 included two minor clusters in which one consisted of Ganesh, Jyoti Nirmala-303 and Shive, while another minor cluster included cultivar Parbhani kranti alone. Sub-cluster B_1 consisted of genotype GO-2, while another sub cluster B_2 contained genotype GAO-5.

4.7.4 Cophenetic correlation study

Correlation study was carried out to compare the correlation of original similarity matrix of SSR results with the dendogram clustering pattern. Using the Cophmodule of NTSYSpc version 2.02, r value was calculated and results were also expressed graphically (Fig 4.9). High correlation between the similarity matrix and dendogram pattern was justified by the r value which was found to be 0.93, and it was very good to fit.

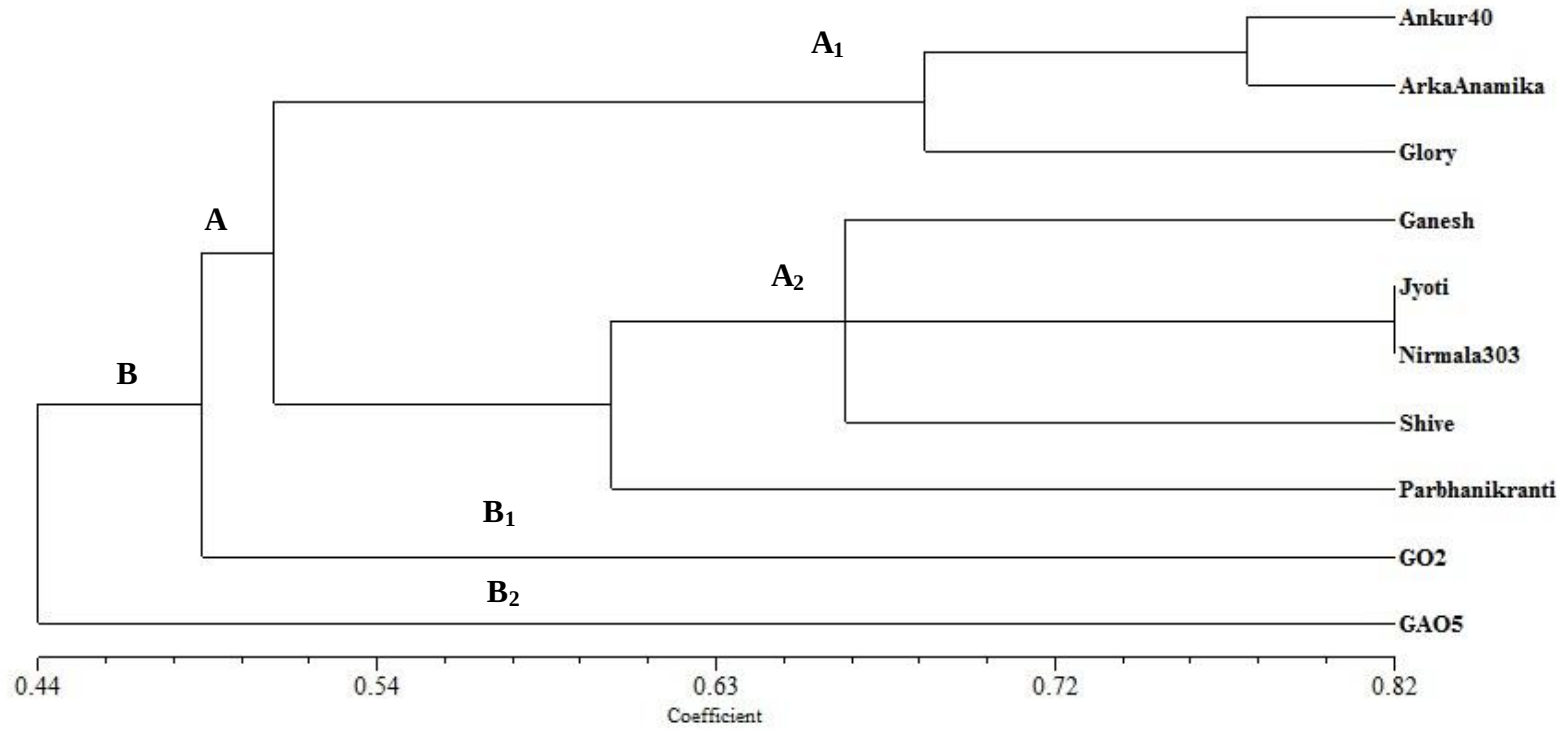

Fig. 4.9: Dendogram showing clustering of 10 okra cultivars constructed using UPGMA based on Jaccard's coefficient obtained from SSR analysis

Table 4.17: Genetic similarity matrix of pooled SSR data based on Jaccard's similarity coefficient

	Ankur-40	Arka Anamika	GAO-5	GO-2	Ganesh	Glory	Jyoti	Nirmala-303	Parbhani kranti	Shive
Ankur-40	1.000									
Arka Anamika	0.778	1.000								
GAO-5	0.455	0.455	1.000							
GO-2	0.455	0.455	0.385	1.000						
Ganesh	0.500	0.500	0.385	0.636	1.000					
Glory	0.778	0.600	0.455	0.600	0.636	1.000				
Jyoti	0.500	0.500	0.636	0.500	0.667	0.500	1.000			
Nirmala-303	0.500	0.500	0.500	0.500	0.667	0.500	0.818	1.000		
Parbhani Kranti	0.500	0.500	0.231	0.385	0.667	0.500	0.538	0.538	1.000	
Shive	0.500	0.500	0.500	0.385	0.667	0.500	0.667	0.667	0.67	1.000

4.8 Correlation studies:

The correlation coefficient of twelve different variables under study was obtained in all ten okra cultivars (Table 4.18).

The results revealed that the plant height was found significantly and positively correlated with fruit length, fruit girth, fruit weight and yield. Whereas plant height, fruit length, fruit girth, fruit weight, and yield showed a highly negative correlation with fruit and shoot infestation with r- value of 0.863, 0.588, 0.583, 0.839 and 0.861, respectively.

The correlation between fruit borer and fruit and shoot borer infestation and biochemical constituents (moisture, total soluble sugars, reducing sugars, non reducing sugars, fiber, antioxidant activity, ascorbic acid, total chlorophyll, chlorophyll a/b, peroxidase activity, polyphenol oxidase activity and catalase activity) was significant. Moisture, total soluble sugars, reducing sugars, non reducing sugars and chlorophyll a/b were significantly and positively correlated with all the two parameters (shoot and fruit damage) of infestation (Table 4.18). Thus, increased in moisture, total soluble sugars, reducing sugars, non reducing sugars and chlorophyll a/b, infestation of fruit and shoot borer increased or vice versa. Fiber, antioxidant activity, ascorbic acid, total chlorophyll, peroxidase activity, polyphenol oxidase activity and catalase activity (Table 4.18) in fruits were significantly and negatively correlated with the infestation of fruit and shoot borer. It indicated that increase in fiber, antioxidant activity, ascorbic acid, total chlorophyll, peroxidase activity, polyphenol oxidase activity and catalase activity in the fruits, infestation of fruit and shoot borer was decrease. Thus, the higher amount of fiber, antioxidant activity, ascorbic acid, total chlorophyll, peroxidase activity, polyphenol oxidase activity and catalase activity was responsible for imparting resistance. Overall, it could be

concluded from the above results that the biochemical constituents *viz.*, lower moisture (%), lower sugars and lower chlorophyll a/b while higher fibrous, antioxidant activity, ascorbic acid, total chlorophyll, peroxidase activity, polyphenol oxidase activity and catalase activity, in okra cultivars provided resistance against fruit and shoot borer infestation.

It could be revealed from the Table 4.18 that moisture was found significantly negatively correlated with carbohydrate, mucilage, fiber, antioxidant activity, total chlorophyll, peroxidase, polyphenol oxidase and catalase, but it was positively correlated with sugars, chlorophyll a/b, and carotenoids.

Table: 4.18 Correlation between morphological, entomological and biochemical attributes

	Plant height	Fruit length	Fruit girth	Fruit weight	Fruit volume	Fruit density	Fruit yield	Shoot borer	Froot& shoot borer	Moisture	Total carbohydrates	Mucilage	Crude fiber	Total soluble sugars	Reducing sugars	Non reducing sugars	Phenol	True protein	Antioxidant activity	Ascorbic acid	Chlorophyll a	Chlorophyll b	Chlorophyll a/b	Total chlophyll	Total carotenoids	Peroxidase	Polyphenoloxidase	Catalase
Plant height	1	.695**	.667**	.915**	.353	.428*	.890**	-.863**	-.863**	-.931**	.523**	.850**	.764*	-.693**	-.550**	-.655**	.149	-.238	.503**	.368*	.415*	.767**	-.635**	.863**	-.867**	.871**	.700**	.518**
Fruit length	.695**	1	.557**	.711**	.416*	.194	.563**	-.588**	-.588**	-.665**	.481**	.750**	.400*	-.618**	-.505**	-.582**	.047	-.297	.226	.158	.058	.598**	-.681**	.585**	-.569**	.600**	.554**	.536**
Fruit girth	.667**	.557**	1	.658**	.238	.324	.620**	-.583**	-.583**	-.647**	.309	.833**	.412*	-.586**	-.432*	-.562**	-.110	.232	.351	.241	.337	.505**	-.434*	.594**	-.592**	.621**	.593**	.466**
Fruit weight	.915**	.711**	.658**	1	.348	.498**	.860**	-.839**	-.839**	-.878**	.539**	.827**	.738*	-.612**	-.687**	-.535**	.232	-.352	.479**	.304	.387*	.723**	-.658**	.844**	-.795**	.738**	.685**	.579**
Fruit volume	.353	.416*	.238	.348	1	-.631**	.262	-.379*	-.379*	-.339	.311	.318	.358	-.124	-.123	-.112	.237	.064	.131	.080	.135	.168	-.279	.229	-.213	.307	.241	-.003
Fruit density	.428*	.194	.324	.498**	-.631**	1	.468**	-.347	-.347	-.404*	.121	.394*	.261	-.415*	-.474**	-.361	-.019	-.311	.280	.172	.222	.420*	-.257	.468**	-.460*	.344	.347	.497**
Fruit yield	.890**	.563**	.620**	.860**	.262	.468**	1	-.861**	-.861**	-.923**	.431*	.749**	.772*	-.611**	-.511**	-.572**	.124	-.289	.517**	.297	.433*	.778**	-.617**	.893**	-.890**	.804**	.700**	.620**
Shoot borer	-.863**	-.588**	-.583**	-.839**	-.379*	-.347	-.861**	1	1.000**	.903**	-.462*	-.761**	-.710*	.657**	.601**	.604**	-.138	.352	-.458*	-.303	-.381*	-.705**	.606**	-.805**	.850**	-.817**	-.739**	-.506**
Froot & shoot borer	-.863**	-.588**	-.583**	-.839**	-.379*	-.347	-.861**	1.000**	1	.903**	-.462*	-.761**	-.710*	.657**	.601**	.604**	-.138	.352	-.458*	-.303	-.381*	-.705**	.606**	-.805**	.850**	-.816**	-.739**	-.506**
Moisture	-.931**	-.665**	-.647**	-.878**	-.339	-.404*	-.923**	.903**	.903**	1	-.601**	-.830**	-.813*	.738**	.557**	.704**	-.010	.292	-.558**	-.309	-.407*	-.762**	.628**	-.862**	.874**	-.850**	-.730**	-.610**
Total carbohydrats	.523**	.481**	.309	.539**	.311	.121	.431*	-.462*	-.462*	-.601**	1	.416*	.560*	-.465**	-.452*	-.422*	-.012	-.086	.220	.104	.184	.527**	-.466**	.551**	-.405*	.373*	.403*	.254
Mucilage	.850**	.750**	.833**	.827**	.318	.394*	.749**	-.761**	-.761**	-.830**	.416*	1	.577*	-.738**	-.585**	-.698**	-.053	-.298	.415*	.203	.350	.616**	-.633**	.722**	-.784**	.779**	.713**	.621**
Crude fiber	.764**	.400*	.412*	.738**	.358	.261	.772**	-.710**	-.710**	-.813**	.560**	.577**	1	-.475**	-.349	-.455*	.035	-.149	.492**	.384*	.452*	.599**	-.469**	.695**	-.686**	.695**	.585**	.437*
Total soluble sugars	-.693**	-.618**	-.586**	-.612**	-.124	-.415*	-.611**	.657**	.657**	.738**	-.465**	-.738**	-.475*	1	.611**	.985**	.191	.323	-.347	-.025	-.342	-.605**	.545**	-.609**	.659**	-.733**	-.635**	-.397*
Reducing sugars	-.550**	-.505**	-.432*	-.687**	-.123	-.474**	-.511**	.601**	.601**	.557**	-.452*	-.585**	-.349	.611**	1	.466**	-.158	.321	-.366*	-.113	-.236	-.476**	.524**	-.546**	.483**	-.453*	-.461*	-.566**
Non reducing sugars	-.655**	-.582**	-.562**	-.535**	-.112	-.361	-.572**	.604**	.604**	.704**	-.422*	-.698**	-.455*	.985**	.466**	1	.248	.292	-.309	-.003	-.331	-.573**	.495**	-.562**	.632**	-.721**	-.610**	-.321
Phenol	.149	.047	-.110	.232	.237	-.019	.124	-.138	-.138	-.010	-.012	-.053	.035	.191	-.158	.248	1	.024	-.015	.184	.182	.099	-.078	.172	.007	-.034	-.016	-.014
True protein	-.238	-.297	-.263	-.352	.064	-.311	-.289	.352	.352	.292	-.086	-.298	-.149	.323	.321	.292	.024	1	-.250	.023	-.154	-.145	.122	-.257	.370*	-.206	-.274	-.133
Antioxidant activity	.503**	.226	.351	.479**	.131	.280	.517**	-.458*	-.458*	-.558**	.220	.415*	.492*	-.347	-.366*	-.309	-.015	-.250	1	.097	.402*	.122	-.035	.287	-.529**	.590**	.386*	.499**
Ascorbic acid	.368*	.158	.241	.304	.080	.172	.297	-.303	-.303	-.309	.104	.203	.384*	-.025	-.113	-.003	.184	.023	.097	1	.107	.140	-.011	.307	-.260	.309	.092	.093
Chlorophyll a	.415*	.058	.337	.387*	.135	.222	.433*	-.381*	-.381*	-.407*	.184	.350	.452*	-.342	-.236	-.331	.182	-.154	.402*	.107	1	.063	.072	.370*	-.440*	.419*	.219	.159
Chlorophyll b	.767**	.598**	.505**	.723**	.168	.420*	.778**	-.705**	-.705**	-.762**	.527**	.616**	.599*	-.605**	-.476**	-.573**	.099	-.145	.122	.140	.063	1	-.884**	.860**	-.662**	.589**	.601**	.500**
Chlorophyll a/b	-.635**	-.681**	-.434*	-.658**	-.279	-.257	-.617**	.606**	.606**	.628**	-.466**	-.633**	-.469*	.545**	.524**	.495**	-.078	.122	-.035	-.011	.072	-.884**	1	-.758**	.540**	-.503**	-.602**	-.510**
Total chlophyll	.863**	.585**	.594**	.844**	.229	.468**	.893**	-.805**	-.805**	-.862**	.551**	.722**	.695*	-.609**	-.546**	-.562**	.172	-.257	.287	.307	.370*	.860**	-.758**	1	-.787**	.732**	.649**	.498**
Total carotenoids	-.867**	-.569**	-.592**	-.795**	-.213	-.460*	-.890**	.850**	.850**	.874**	-.405*	-.784**	-.686*	.659**	.483**	.632**	.007	.370*	-.529**	-.260	-.440*	-.662**	.540**	-.787**	1	-.825**	-.741**	-.556**
Peroxidase	.871**	.600**	.621**	.738**	.307	.344	.804**	-.817**	-.816**	-.850**	.373*	.779**	.695*	-.733**	-.453*	-.721**	-.034	-.206	.590**	.309	.419*	.589**	-.503**	.732**	-.825**	1	.798**	.511**
Polyphenoloxidase	.700**	.554**	.593**	.685**	.241	.347	.700**	-.739**	-.739**	-.730**	.403*	.713**	.585*	-.635**	-.461*	-.610**	-.016	-.274	.386*	.092	.219	.601**	-.602**	.649**	-.741**	.798**	1	.544**
Catalase	.518**	.536**	.466**	.579**	-.003	.497**	.620**	-.506**	-.506**	-.610**	.254	.621**	.437*	-.397*	-.566**	-.321	-.014	-.133	.499**	.093	.159	.500**	-.510**	.498**	-.556**	.511**	.544**	1

5. SUMMARY AND CONCLUSION

The investigation on "Morphological, Biochemical and Molecular characterization of okra cultivars (*Abelmoschus esculentus* L.)" was carried out at the Department of Biochemistry, BACA, Main Vegetable Research Station and Department of Biotechnology, AAU, Anand during 2013-2014.

Okra (*Abelomoscus esculentus* L.) is an important vegetable crop in India with the highest production in the world. Gujarat produces 10 % okra production of total Indian production.

Growth and yield attributes like plant height, fruit length, fruit girth, fruit weight, fruit volume, fruit density, and fruit yield were measured. Significantly the highest plant height was recorded for genotype GAO-5 (101.87 cm). Higher fruit length was observed in GAO-5 (13.70 cm) which was statistically at par with GO-2 (13.56 cm). Fruit girth and fruit weight were higher in GAO-5. The fruit volume of okra fruit was also higher in GAO-5 (9.28 cm^3) followed by Parbhani kranti (8.70 cm^3), Jyoti (8.68 cm^3) and Nirmala-303 (8.67 cm^3). Fruit density was higher in GO-2. Fruit yield was also the highest in GAO-5 (85.89 q/ha) as compared to other genotypes. This might be due to its genetic makeup and more statistically towards infection.

The population of fruit and shoot borer (35 DAS) and fruit borer (60 DAS) were recorded among different okra cultivars. The results showed the least population in genotype GAO-5 and the maximum in cultivar Glory. GAO-5 was observed with higher phenol and fiber content, this might be reason for resistance against insects.

Different biochemical components were measured from fruits of okra. The okra fruits had 73.55-89.66 percent moisture, 6.77-9.03 percent total carbohydrates,

188-246 mg/100gm mucilage, 0.433 – 1.067 mg/gm fiber, 2.31 -2.90 total soluble sugar, 0.44 - 0.55 percent reducing sugar, 1.87–2.35 percent non reducing sugar, 0.055– 0.086 percent phenol, 1.64– 1.97 percent protein, 0.35 – 0.49 percent total antioxidant activity, 36.44 – 39.66 mg/100gm ascorbic acid, 1.17 mg/gm total chlorophyll, 0.85 – 0.98 mg/gm chlorophyll a, 0.26 – 0.55 mg/gm chlorophyll b, 1.67 – 3.47 chlorophyll a/b ratio and 0.038 – 0.085 mg/gm carotenoids. Total soluble sugars was recorded lower in GAO-5 which suggested that lower concentration of sugar present in resistant genotype would be playing a role in signaling for disease resistance.

Peroxidase, polyphenol oxidase and catalase activities were recorded the maximum in GAO-5 (17.84, 11.40 and 0.627 min^{-1} g^{-1}fw respectively) and the minimum in Glory (14.59, 9.56 and0.627Δ O.D. min^{-1} g^{-1}fw respectively). Peroxidase did not play direct role in resistance against pathogen but plant disease resistance had often been correlated with elevated peroxidase activity and the oxidation of phenolics in diseased tissues. The role of peroxidase in plant defense mechanism had been attributed to its ability to oxidize key metabolites in plant or pathogen (Chittoor*et al.*, 1999). Peroxidase was also involved in lignin synthesis and degradation of cytotoxic levels of hydrogen peroxide enerated in plant tissues as a result of pathogen attack (Van loon, 1997). The PPO specific activity induced in resistant okra genotype could be a defensive response against YVMV infection and seemed to be related to disease resistance. PPO seemed to be compartmentalized in plant cells but as a result of pathogenic attack or during tissue senescence, membrane disruption could occur, which could initiate formation of quinones following an increase in accessibility of PPO to its substrate (Mohammadi and Kazemi, 2002).

Fruit protein of okra cultivars was analyzed through electrophoresis. Variation for the protein banding pattern and band intensity were observed in all the cultivars. Most of the cultivars could be identified on the basis of specific banding pattern and the presence or absence of bands in respect to specific cultivar for fruit protein. The Jaccard's similarity coefficient of protein profile ranged from 0.40 to 1.00. Dendogram revealed formation of two main clusters depicted as A and B. The minimum similarity was observed between Ankur-40 and Glory, whereas the maximum similarity was observed between Nirmala-303 and Parbhani kranti, and Ganesh and Shive.

The protein electrophoresis is a rapid technique useful to differentiate genotypes. Variation in banding pattern and band intensity could be used for varietal identification. Protein which was encoded at several loci within genome exhibited extensive polymorphism, both in relation to size and charge.

The isozyme pattern of peroxidase, polyphenol oxidase and super oxide dismutase showed presence or absence of different isoforms. The number of isoforms were more in peroxidase and super oxide dismutase, while less in polyphenol oxidase.

The peroxidase isozyme showed 6 isoforms with Rm values ranged from 0.135 to 0.497. The similarity index for peroxidase was in the range of 0.5 to 1.000. The polyphenol oxidase enzyme showed 3 bands with Rm values ranged from 0.120 to 0.344. The Jaccarrd's similarity index (SI) of polyphenol oxidase isozyme ranged between 0.33 to 1.00.

The isozyme pattern of superoxide dismutase revealed the presence of 5 to 6 bands with Rm values ranging from 0.085 to 0.544. The similarity index for SOD ranged between 0.6667 to 1.000 value. Among all three isozyme studied, SOD pattern was most complex and showed maximum polymorphism among the cultivars.

For present study peroxidase, superoxide dismutase and polyphenol oxidase showed two different main clusters as A and B. Thus, it could be concluded that, okra cultivars resided under main cluster A were genetically far from the genotypes, which felt under main cluster B, but genotypes included within main and sub-clusters were genetically similar. Isozyme provide interesting information for species differentiation, but their use seems less appropriate for studies of within species genetic variation.

The isozyme of peroxidase showed differential accumulation of peroxidase isoforms amongst resistant and susceptible entries and could be differentiated by two unique bands having Rm value 0.454 and 0.471. PPO banding pattern was able to discriminate between resistant and susceptible genotypes.

In the SSR analysis 8 markers were amplified and produced 19 alleles. The average number of alleles per locus was found to be 2.37, while effective number of alleles was 1.49. The maximum number of alleles was 7 which were recorded for markers OK – 2, whereas OK - 6 produced four alleles. The highest PIC value was recorded for OK-5 (0.60), where as the lowest value was recorded for OK-1 (0.48). The average PIC value was 0.81 recorded for OK-2. The molecular weight of the amplified PCR products ranged from 143 to 557bp. The highest similarity index value 0.82 was found between Jyoti and Nirmala-303; whereas, the least similarity index value 0.23 was found between GAO-5 and Parbhani kranti.

Dendogram based on Nei's 1978 unbiased measures of genetic distance by UPGMA clustering algorithm method grouped the cultivars into two main clusters designated as A and B.

According to present investigation it could be concluded that among ten okra cultivars the GAO-5 was superior variety, due to its resistance against fruit and shoot

borer, high carbohydrates, fiber, phenol, antioxidant activity, ascorbic acid, total chlorophyll, peroxidase, polyphenol oxidase and catalase activity.

Further study need to carried out on individual amino acid, which would provide a clue to understand the mechanism of plant tolerance.

Relationship between allozymes of an antioxidative enzymes and crop production may open a new era.

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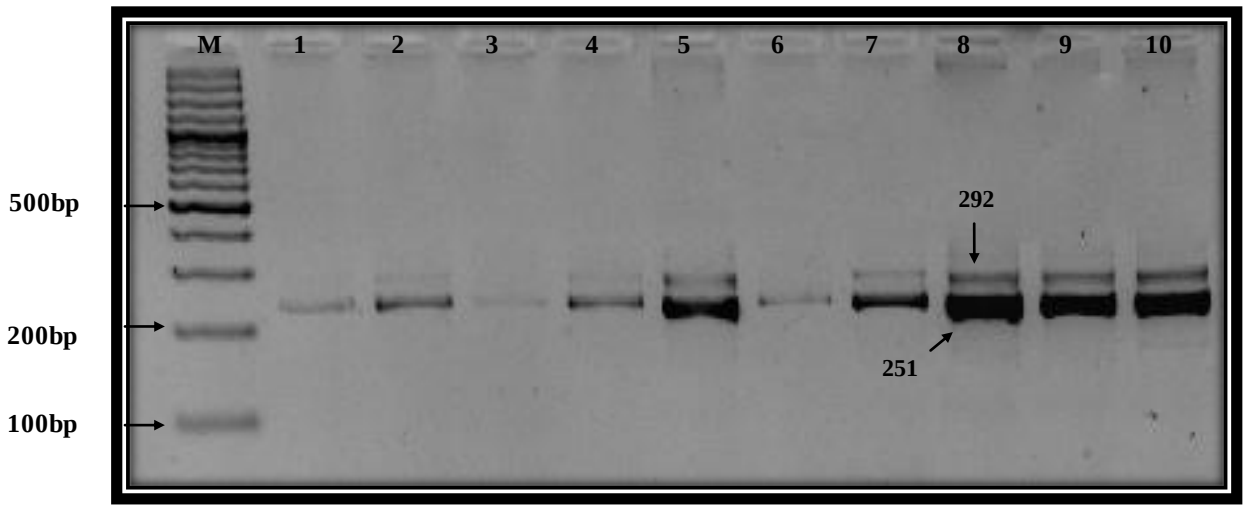

Plate 4.5: SSR profile of OK-1

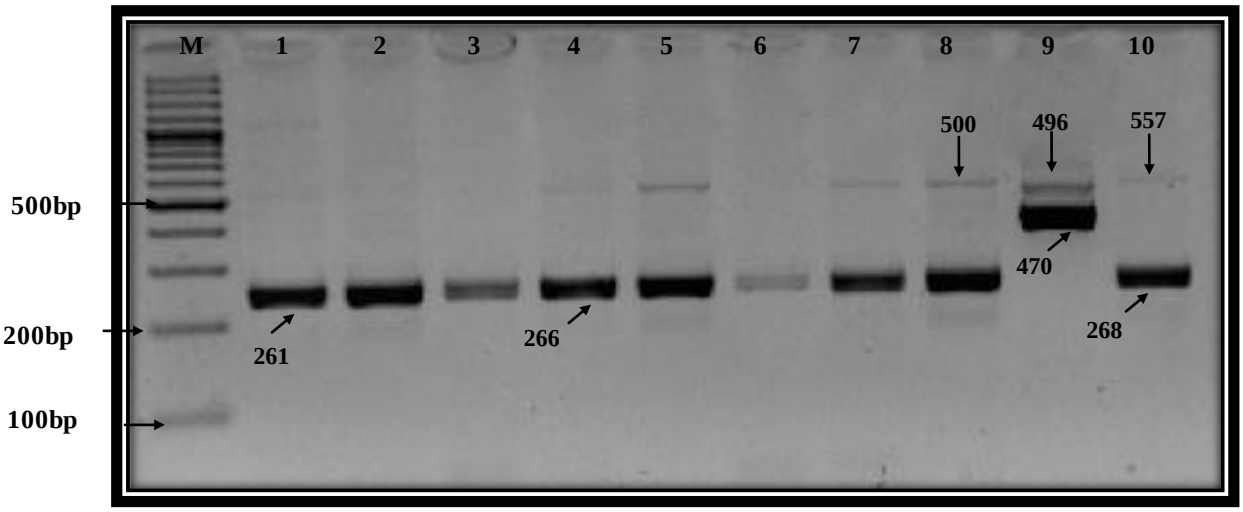

Plate 4.6: SSR profile of OK-2

M = Protein marker (1) Ankur-40 (2) Arka Anamika (3) GAO -5 (4) GO-2 (5) Ganesh (6) Glory (7) Jyoti (8) Nirmala-303 (9) Parbhani kranti (10) Shive

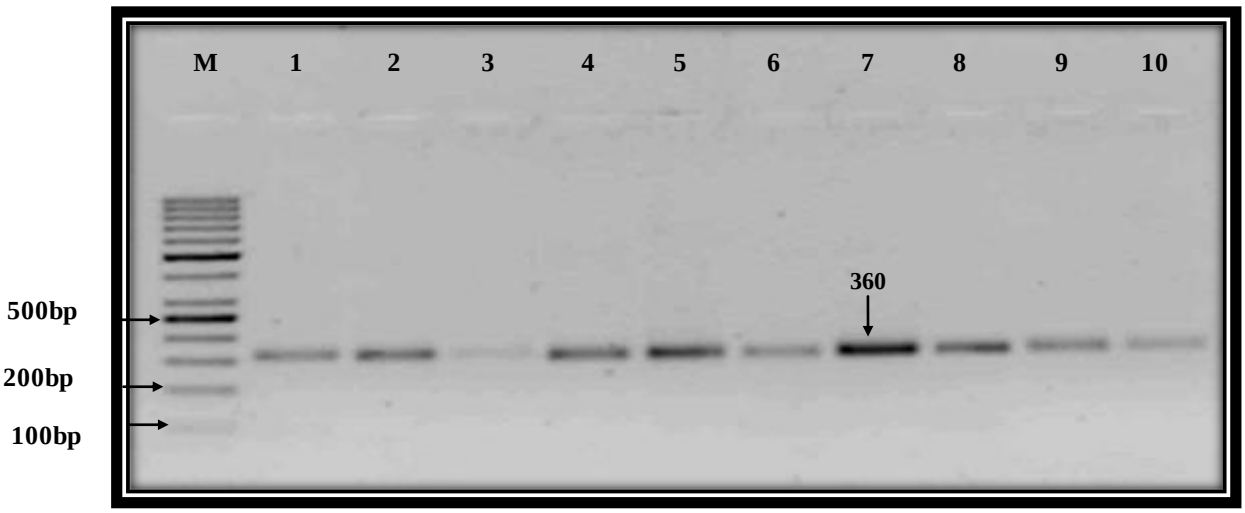

Plate 4.7: SSR profile of OK-3

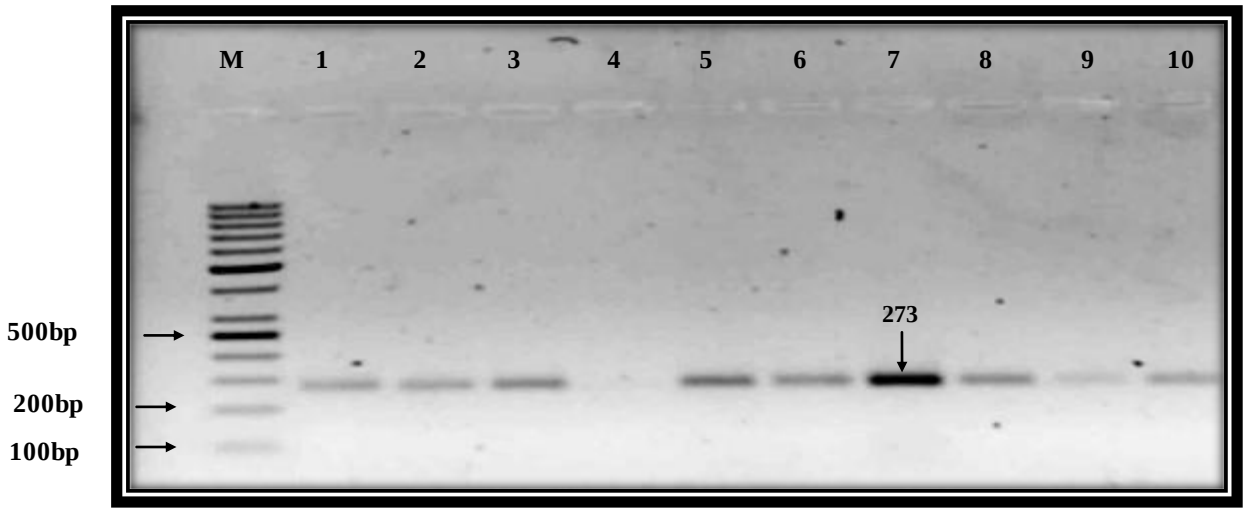

Plate 4.8: SSR profile of OK-4

M = Protein marker (1) Ankur-40 (2) Arka Anamika (3) GAO -5 (4) GO-2 (5) Ganesh (6) Glory (7) Jyoti (8) Nirmala-303 (9) Parbhani kranti (10) Shive

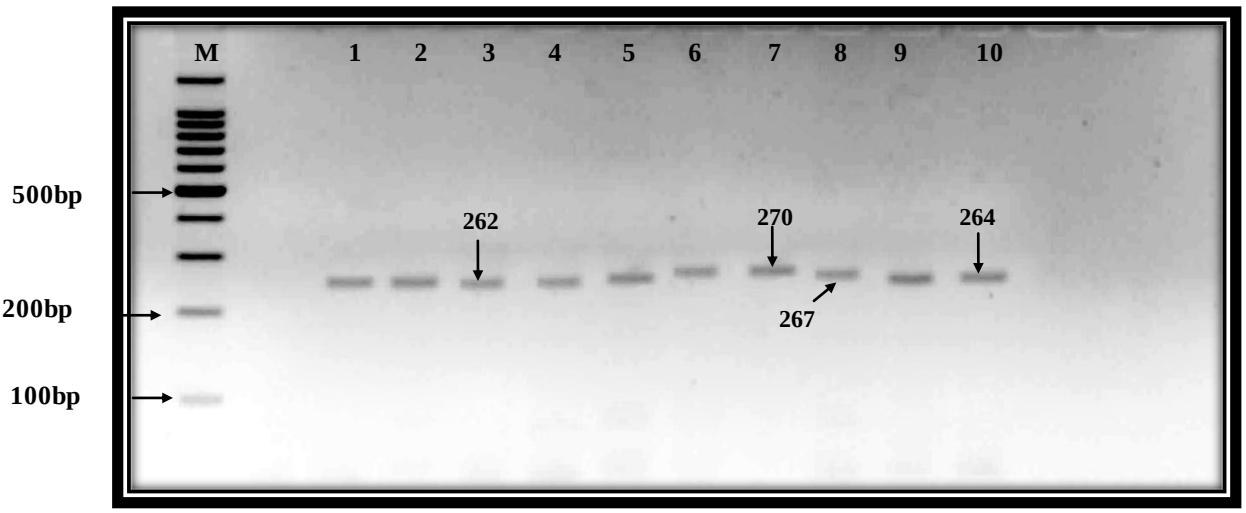

Plate 4.9: SSR profile of OK-6

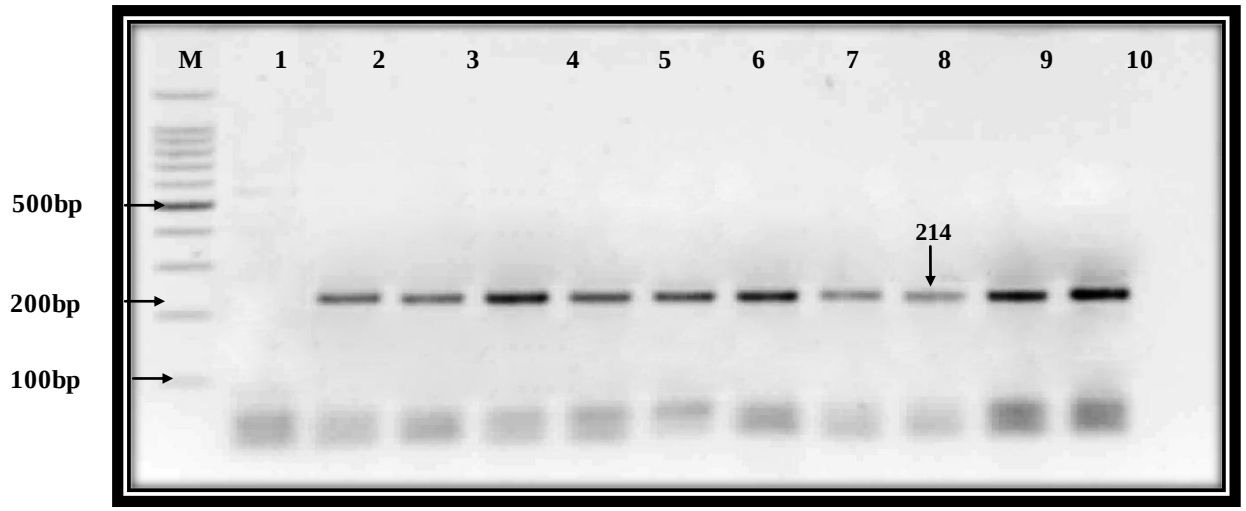

Plate 4.10: SSR profile of OK-7

M = Protein marker (1) Ankur-40 (2) Arka Anamika (3) GAO -5 (4) GO-2 (5) Ganesh (6) Glory (7) Jyoti (8) Nirmala-303 (9) Parbhani kranti (10) Shive

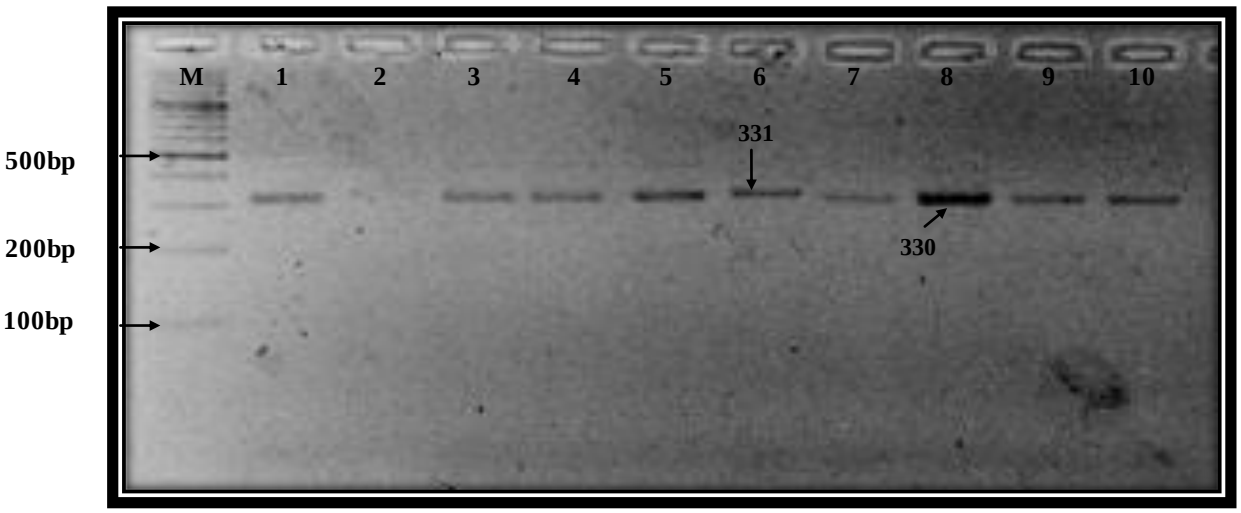

Plate 4.11: SSR profile of OK-9

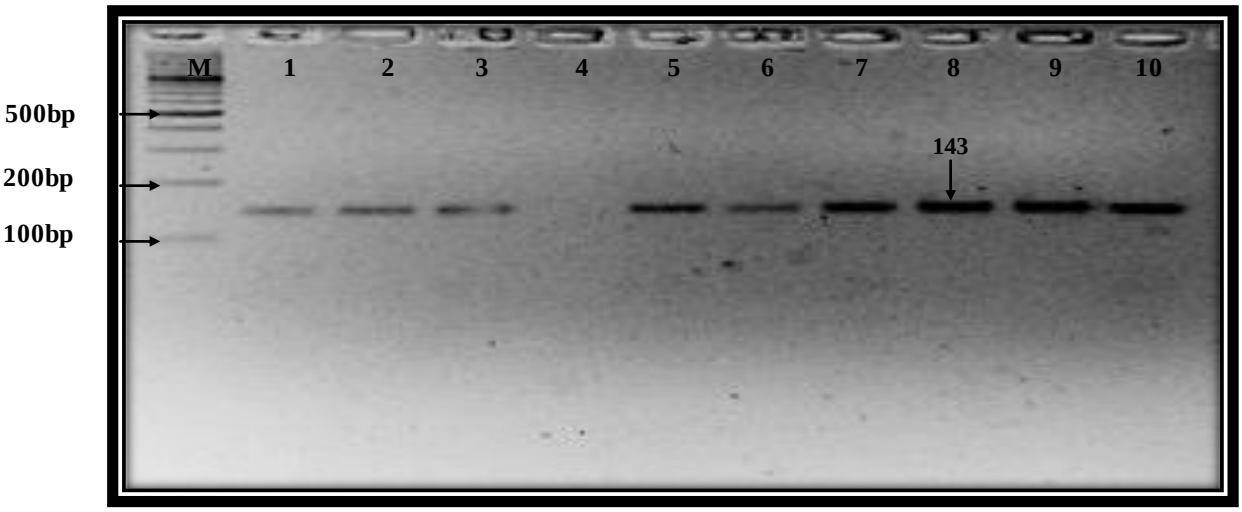

Plate 4.12: SSR profile of OK-12

M = Protein marker (1) Ankur-40 (2) Arka Anamika (3) GAO -5 (4) GO-2 (5) Ganesh (6) Glory (7) Jyoti (8) Nirmala-303 (9) Parbhani kranti (10) Shive