Heterosis for yield and yield components in okra genotypes

DISSERTATION- II

SYNOPSIS

Submitted by:

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In partial fulfillment for the award of the degree
Of
Masters of Science in agriculture
(Genetics and Plant Breeding)

Under Guidance of
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CERTIFICATE

This is to certify that the project entitled study **of "Heterosis for yield and yield components in okra genotypes"** is currently performing by Sreeparna Chowdhury (11706623), as per the research work (dissertation program) GPB 596 in partial fulfillment for the award of the degree of Masters Of Science in Agriculture (Genetics & Plant Breeding) from Lovely Professional University, Phagwara, Punjab under the guidance of supervisor.

Dr. Sanjeev Kumar

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DECLARATION

I declare that this report is an overview of practical work which I am currently performing. It is my individual research under the project entitled " **Heterosis for yield and yield components in okra genotypes** " going to complete during the academic period 2017-2019 of M.sc. Agriculture (Genetics & Plant Breeding) course, supervising and guiding by Dr. Sanjeev Kumar, assistant professor at Lovely Professional University, Phagwara, India.

I also declare that this project will be done in this university only and it shall not be submit to any other university for the award of any degree.

Sreeparna Chowdhury (11706623)

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INTRODUCTION

Okra (*Abelmoschus esculentus* (L.) Moench) generally known as "Lady's finger', 'Gumbo' or 'Bhindi', is one of the important vegetable crop of India. Its cultivation ranges throughout the tropical and sub-tropical zones and also in the hottest areas of the temperate zones of world for its fibrous edible fruits or pods. Okra is polyploidy in nature having chromosome number 2n=130 or other series of 2n= 72, 108, 120, 132 or 144 with n=12 (Dutta and Naug, 1968) and lies in the family of Malvaceae. The origin of Okra is near Ethiopian region and during the course of evolution its spread out to North African and Middle East part of the world (Lamont W 1999, Tindall H. D 1983). It is one of the heat tolerant vegetable crops grown worldwide but prefers temperature ranges between 20° - 35°C. It is grown on a variable types of soil included the soil should have adequate drainage property with good amount of organic content (Akinyele B.O. et.al. 2007). Optimum pH of 6-6.5 is reported to be suitable for *Abelmoschus esculentus* cultivation. Considering world scenario, okra is grown in the area of around 1148 thousand hectare with the production of about 7896.3 thousand tonnes. Countries involves in okra cultivation are India, USA, Nigeria, Mexico, Pakistan, etc. Among all countries, India holds the 1st rank for okra production with 5,784 thousand tonnes that constitute 72% of overall world production. In case if India, Andhra Pradesh is the leading producer of okra followed by West Bengal in terms of area, production and productivity (78.9 thousand hectares of area, production of approximately 1184.2 thousand tonnes with a productivity of 15 tonnes/hectare and area of 74 thousand hectares, production of 862 thousand tonnes with a productivity of 11.70 tonnes/hectare respectively).

Okra is an annually growing plant having life cycle of 90 to 100 days, multiplied using seeds generation after generation. It s having erect stem with branching, leaves are of alternate pattern whereas has auxiliary flowers. It grows in variable height ranges from 0.5 to 4 meters. Okra is an often cross pollinated crop generally assisted by insects and extent of cross pollination ranges from minimum 4% to maximum 42 percent (Kumar, 2006). It is cultivated for its edible and fresh fruits or pods which contain very good amount of nutrients. In 100g of fresh and mature okra pods contains 6.4 g carbohydrates, 1.9g of protein, 3100 cal energy, 0.2 g fat, 1.2 g fibers ,

0.8g minerals and other vitamins like A,B and C. Okra is a potential foreign exchange crop accounts for almost 60% of the total fresh vegetables export from India (Rewale et al., 2003).

Okra also contain mucilage (1.6 g /100g of pod) i.e. thick and slimy substance present in fresh pods. It is actually polysaccharide, acidic in nature associated with proteins and other biomolecules (Woolfe et al., 1977). Okra mucilage has medicinal property and can also be utilized for food and non-food products.

Our country has diverse climatic conditions leads to the existence of variable numbers of okra cultivars showing wide variations among there qualitative and quantitative characters. Although okra is cultivated from past many years, still one of the significant problems with its production is non availability of appropriate location specific high yielding cultivars. Therefore the main aim of present study to exploit hybrid vigor ability of okra to produce hybrids that can further be utilized for crop improvement programme (Kumar et. al., 2006).

Conventional method of analyzing heterosis is being going on several decades till now. Marphological analysis is always been an effective method for knowing the magnitude of heterosis but the major constraint is that the marphological data can be alter due to environmental factors. So now in the century of bimolecular advancement, studies including molecular analysis along with conventional method became the best way for confirming the magnitude of heterosis.

More recently, DNA markers have been reliably used in cultivar identification (Moser and Lee 1994), diversity analysis (Vasconcelos et al. 1996), construction of genetic maps (Song et al. 1991) and tagging agronomical important genes (Kelly 1995). Application of DNA markers is the prediction of heterosis in hybrids. Evaluation of hybrids for heterosis or combining ability in the field is expensive and time-consuming. As a result, many parameters such as pedigree information, qualitative and quantitative traits (Smith et al. 1990; Wang et al.1992) and biochemical data (Leonardi et al. 1991) are used to study heterosis. In *Abelmoschus esculentus*, utilization of various molecular markers such as RAPD, SSR, EST-SSR and ISSR are more effective way of heterosis confirmation when congruent with phonological characters. Biochemical methods like protein analysis and isoenzymes are also utilized for analyzing quality parameters of different okra varieties.

In past, most of the improvements in okra were generally based on selection process or through hybridization for location adaptation and heterosis. Recently, from few years back hybridization technique along with the incorporation of molecular technology are extensively utilized for achieving better heterosis and combining ability among the desired parents.

OBJECTIVES AND HYPOTHESIS:

The main objective of this research is to test the response of hybrids for quantitative and qualitative traits when crosses were made between different parents combination in okra.
Present study hypothesize that F1 from the crossing among selected okra cultivars (12X12 half diallel) could show better, moderate or low heterosis and combining ability for certain traits.

Based on the assumption and hypothesis, the objectives of this research are mentioned below:

1. To perform crossing in selected parents using suitable mating system.

2. Estimation of combining ability of parent cultivars.

3. Selection and screening of heterotic crosses utilizing molecular and biochemical markers.

REVIEW OF LITERATURE

Utilization of successful heterosis for various characters in crop improvement programme is the one of the main motive of genetics and plant breeding studies. By keeping that in view, the present literature signifies the summary of the research work going on in okra varieties related to my objectives has been highlighted under the following titles:

1) Analysis of heterosis

2) Combining ability

3) Molecular markers

4) Biochemical attributes

1) Analysis of heterosis:

Mehta et.al., (2008), used three testers and fourteen lines of okra cultivars and made forty two crosses among them for studying heterosis and gene action of F1 s along with their parents. In this study various marphological traits like first flowering time, days to 50% flowering, weight of fruit, plant height,yield per plant, etc. were considered. The result shows the most better performance were of Kaveri Selection x Ankur Abhaya ,VRO-6 x Parbhani Kranti, , Daftari-1 x Arka Abhaya and VRO-4 x Parbhani Kranti for fruit yield /plant.

Kumar et.al., (2010), investigated suitable parents with superior parental combination of crosses for yield improvement among okra cultivars. Diallel mating system (with reciprocal) were used to crossed among 6 cultivars i.e. Girija Vikas, MDU 1, Hissar Unnath, Arka Abhay and EC 305623.Outcome showed maximum standard heterosis achieved in MDU 1 x Hissar Unnath, of 65.23 per cent for yield/plant.

Lyngdoh et.al., (2013), conducted experimental trial for identifying heterosis in near isogenic lines if okra cultivars using 18 lines and 4 testers s (Parbhani Kranti, Arka Anamika, VRO-5 and VRO-6) and there 72 F1s were compared against commercial check variety ((MHY-10). Result

revealed maximum amount of heterosis for KO-6 × PK (-43.05%) for internodal length, for plant height is in KO-2 × PK (48.20%), KO-2 × AA (23.90%) for number of leaves, over the better parent and KO-6 × PK (56.07%) for plant height over commercial check variety.

Nagesh et.al., (2014), conducted breeding approaches using 21 parents and commercial check for estimating the magnitude of heterosis in okra genotypes for better quantitative and qualitative parameters. 54 F1s were made by utilizing line X tester mating fashion and analyzed for significant heterosis in compare with commercial check. The study shows relatively good scope of exploitation of hybrid vigor for commercial production of okra.

Patel R.K., (2015), revealed the high magnitude of heterosis in okra for fruit yield and its other related components in a (17X 4) line x tester fashion of mating . The result estimation showed variance in parents and hybrids for major of the traits were significant unlike fruit girth and pod length, which signifies expression of hybrid vigor for these characters. It outlays that combination of VRO X PK and M-65 X GO-2 exhibit most superior heterotic F1s for yield and some other related parameters.

Patel BG and Patel AI, (2016), studied was to estimate level of heterosis for 12 characters in okra by applying line X tester design. In the study, total 45 genotypes *viz*. eight lines, four tester, thirty two F1s from cross combination along with one check cultivar were analyzed . Genetic variation is observed among the hybrids confirming by the presence of significant variance among parent cultivars and their F1 progenies. Cross combination of JOL-10-17 x GJO-3, AOL-10-18 x VRO-6, JOL-09-8 x PUSA SAWANI and JOL-09-7 x PUSA SAWANI were recorded with significant amount of standard heterosis for fruit yield.

Maciel et.al. ,(2017), assessed 2 inbred lines (obtained by selection of three cycles) *viz.* UFU-QB-040D and UFU-QB-107G for estimating heterosis in okra. Two methods of hybridization i.e. traditional (complete emasculation along with artificial pollination) and experimental method (incomplete emasculation along with insect pollination) were compared for obtaining heterotic hybrids for hybrid seed production. The resultant outcome revealed the magnitude of heterosis in experimental hybridization technique shows almost similar results as that of traditional technique.

2) Combining ability:

Wamm et.al., (2010), studied 9 okra genotypes by applying 9 X9 diallel mating pattern for yield and its related parameters. The evaluation of 9 parents along with their 36 hybrids revealed significant variance in general combining ability (GCA) and specific combining ability (SCA) for all characters undertaken. It signifies that the characters are governed by both additive and non additive gene effect. Its concluded in the study that combining ability and heterotic behavior of the parents and hybrids respectively can be governed by mean performance of them.

Raghuvanshi et. al., (2011), analyzed combining ability in 10 okra genotypes utilizing line X tester (6 X 4) method. Significant magnitude of GCA and SCA were observed for all selected characters signifies the gene action of both additive and non- additive types. Different parents were found to be good combiners of various different characters *viz.* HRB-55 (intermodal length), HRB-9-2 and VRO-6 (first flowering, pod length, yield/plant) whereas 3 cross combination were found showing SCA for fruit yield/plant *viz.* HRB-9-2 X Arka abhay, HRB55 X Arka abhay and HRB-9-2 X P-7.

Adiger et. al., (2013), estimated combining ability of 43 cultivars of okra for various traits. The experiment generated 120 crosses by using line X tester mating system having 40 female lines and 3 testers. Outcome showed higher SCA variance than GCA, implies presence of non- additive gene effect for almost all traits. The genotype Prabhani Kranthi was reported as having good combining ability for parameters like plant height, branches/plant, fruit weight, pod length, yield pod/plant and yield/area which indicate the chances of utilization of this genotype in breeding programme for crop improvement.

Kumar et.al., (2015), undergone an experiment with 12 parental lines in okra and studied all parental lines and there F1 hybrids generated by half diallel mating fashion for estimating the magnitude of combining ability. Total of 17 characters were taken into consideration and observed the presence of significance level of GCA and SCA for all these characters. Good combiner parents were Hisar Unnat for fruit yield, IC- 1288891 for earliness and VRO-5 for pod length and diameter, and Larm-1 × IC-111527, IC-282280 × IC-111527 and IC-282280 × EC-

329380 were the combinations signifying SCA for characters like earliness, yield per plant, etc. The result revealed the scope for utilization of these parents in further crop breeding programmes.

Kumar and Thirupathi, (2016), reported the combining ability among the diallel crosses of six inbred lines (RNOYR-14, RNOYR-15, RNOYR-16, RNOYR-17, RNOYR-18 and RNOYR-24) of okra. Analysis showed the predominance of both additive of non- additive gene action governed by the observation of highly significant magnitude of GCA and SCA for almost all the selected characters. Parental combination of RNOYR-17×RNOYR-18, RNOYR14×RNOYR-17 and RNOYR-16×RNOYR-17 showed promising SCA for total fruit whereas RNOYR-16 was depicted as the best GCA.

3) <u>Molecular markers:</u>

Saifullah et.al., (2010), examined molecular phylogeny and diversity in 121 okra cultivars employing RAPD markers. DNA profiling with 5 primers out of 39 generated 38 visible bands, out of which 32 bands showed polymorphism *viz*. 6.4 diversity/bands. UPGMA was utilized to produce dendogram of 121 genotypes representing 8 cluster groups based on diversity. Observation reported to have value of 0.54 for the correlation between marphological and molecular data. The study concluded the presence of broad genetic base of genotypes and revealed the possibility of utilization of RAPD markers for analyzing germplasm source.

Khan et.al., (2013), studied RAPD markers employed genetic diversity among 39 okra cultivars. 21 polymorphic primers produced 111 amplified bands (5.5 fragments / primer), out of them 107 were observed polymorphic constituting 96% of the total fragments. The resultant dendogram analysis had shown grouping of genotypes in 7 clusters with maximum closeness of 83% between genotypes (Sabzpari 2001 and Acc.No.019221) and minimum of 44.14% among the genotypes (Acc.No.019217 and Punjab Selection) emphasizing the presence of variability in the okra genotypes.

Schafleitner et.al.,(2013), studied combined leaf and pod transcriptome of okra obtained from RNA sequencing. Okra transcriptome sequences were mined for simple sequence repeat (SSR)

markers. Among 161 polymorphic SSR markers, 19 were selected for genetic diversity analysis on 65 genotypes of three different species. The result shows the clustering of genotypes depending upon geographic origin. This study provides with gene sequence information and the markers of okra which were made available for further breeding research.

Yuan et.al., (2014), investigated 24 okra lines for genetic diversity by exploiting 22 ISSR markers. Following procedure of PCR, electrophoresis and visualization under silver staining; viewed 289 amplified DNA bands generated by the 22 primers among which 50% were polymorphic. Cluster estimation based on UPGMA revealed a dendogram of 4 groups of genetically diverse genotypes. The PIC result of 0.531929 showed that almost all primers were instructive and can successfully be utilize in the studies related to diversity.

Yıldız et.al., (2015), studied 66 okra genotypes applying iBPS- retrotransposon markers for diversity analysis. The study showed 88 DNA bands with polymorphism level of 40.2%, PIC value were 0.12 to 0.99 and 0.52 to 0.81 for retrotransposons and SSR respectively. Combine analysis of retrotransposon and SSRs grouped the genotypes in four clusters and population structure was estimated among the cultivars with the help of STRUCTURE software. The outcome depicted that SSR markers were effective in clustering the genotypes into groups and also the efficient use of iPBS - retrotransposons for diversity analysis.

Helaly et.al., (2017), analyzed nine okra cultivars for genetic components utilizing DNA fingerprinting techniques. The study observed landraces from geographical diverse regions and by incorporating RAPD-PCR method along with 6 primers. It shows total of 61 amplified bands as as a resultant which further differentiated as seven monomorphic bands (i.e. 11.47%), one negative unique band constitute 1.63% of the total, twelve positive unique bands (19.67%) and forty two polymorphic bands i.e. of 68.58%. Further the dendograms were estimated and revealed that the cluster B divided to 2 sub cluster ‘c’ and‘d’ signifies presence of good magnitude of polymorphism . It was concluded that the cultivars have genetic variability among them that can be utilized in crop improvement programmes for the development of new lines with better characters.

4) Biochemical attributes;

Osawaru et.al., (2012), characterizes 9 genotypes of okra based on seed protein and marphological parameters. Protein analysis were done using SDS- PAGE and 4 protein bands each for one genotype was obtained whereas none of the band appeared for other 5 genotypes.

Dhruve et.al., (2015), studied biochemical parameters of okra considering 10 genotypes *viz.* AOL 10-22, AOL 13-75, GAO 5, , AOL 13-90, AOL 12-55, AOL 13-73, AOL 13-88, pusa sawani, Prabhani kranti and a wild cultivar. Parameters like protein content, oil content, mucilage, total soluble sugars, carbohydrates, etc. were evaluated and found that variety GAO 5 have highest protein content (18.96%) and highest oil content (14.40%) whereas total carbohydrates was highest in AOL 13-90 (29.82%).

Castillo et.al., (2017), reported bioactive peptides extracted from crude protein of okra seeds which is having antihypersensitivity property. Okra seed crude protein extract (OSCPE) was extracted which was estimated as 13.8 ± 0.6 mg/ml. OSCPE were further examined by using SDS- PAGE to reveal the presence of polypeptide bands and bioactive peptide were obtained through enzymatic hydrolysis.

Helaly et.al., (2017), examined nine Egyptian okra cultivars for molecular variability. Analysis of the seed protein was estimated using Sodium Dodecyl Sulfate Poly Acrylamide Gel Electrophoresis (SDS- PAGE). The outcome showed total of 124 protein bands on the gel with molecular weight ranges from 206KD to 33 KD. Seed storage protein analysis signifies presence of close relation among the landraces i.e. AlHemaa and Al-Sabahia, Aswan and Red, Ityei El-Barud and Tanta, Damanhur and Al-Sheikh Makram.

MATERIALS AND METHODOLOGIES

A) STUDY AREA:

The present investigation is undergoing in the "Agricultural Research Field, School of Agriculture, Lovely Professional University, Phagwara, Punjab -144411.

1) Geographical situation:

The Lovely Professional University is situated in the south of NH-1 (G.T. Road) in the district Jalandhar of state of Punjab at a distance of about 350 km from Delhi. It is situated between 31o15' North latitude, 75o 42' East latitude at an altitude of 228 meters above the mean sea level (MSL). The total agriculture land of the district is 134 hectare. Major source of irrigation in tube wells, bore wells and then pump sets. Only 3% of land is irrigated by canal.

2) Climatic conditions:

The temperature reaches above 40 degree Celsius during summer and during winter the temperature goes down below 10 degree Celsius. The area falls under sub humid region. June being the hottest month and January being the coldest month. The average rainfall is 719mm. the highest rainfall is recorded during the month of July and the driest month is November.

B) EXPERIMENT DETAIL:

1) Genotypes:

Total of 12 genotypes were used for the ongoing research work.

Table No.1

Serial No.	Genotypes/ Accessions	Details	Source
1	IC 013664	-	NBPGR
2	IC 014026	-	NBPGR
3	EC 305615	-	NBPGR
4	IC 010265	-	NBPGR
5	AKO 107	-	NBPGR

6	EC 305768	-	NBPGR
7	EC 359637	-	NBPGR
8	Rajini		
9	Arka Anamika	*A.esculentus*X*A. manihot ssp. tetraphyllus* (wild type)	IIHR
10	Tropical Bhushan	-	-
11	Annika	Selection variety	Pvt. Company
12	Ankur 40	F1 hybrid	Pvt. Company

2) **Experiment details:**

The experimental material of present study comprised of 12 okra genotypes from which 66 hybrids are expected to obtained from half diallel mating design (12 x 12). The crosses are going to be made during *summer* 2018-19 which going to be evaluate during *kharif* season 2018-19 along with parents under the field condition of Lovely Professional University, Phagwara, India.

Table No.2

1st season (crossing)	
Crop	Okra (Abelmoschus esculentus)
Mating design	Half Diallel Design
Genotype	Accessions and varieties of okra
Parents	12
Experimental year/date of sowing	2018-19 (29 March)
Spacing	60cm X 45cm
Season	*Summer*
Ferti lizer dosage	N: P: K @ 100: 50: 50 kg/ha

2nd season (evaluation)	
Number of genotype	Total 78 (hybrid 66, parent 12)
Design	RBD (Randomize Block Design)
Replication	3
Experimental year	2018-19

3) **Molecular and biochemical analysis:**

- **Following steps to undergo under molecular analysis for genetic diversity:**
- DNA extraction from all samples of 12 genotypes along with 66 hybrids using CTAB method.
- DNA quantification using UV spectrophotometer.
- DNA amplification in PCR using SSR marker.
- Gel electrophoresis and evaluating bands.
- Dendogram formulation.

Table No.3: Details of the SSR markers

Markers	Forward primers	Reverse Primers
AVRDC-Okra1	ATGGAGTGATTTTTGTGGAG	GACCCGAACTCACGTTACTA
AVRDC-Okra8	TGCTGTGGAAGGTTTTTACT	ATGACGAAAGTGGTGAAAAG
AVRDC-Okra9	ACCTTGAACACCAGGTACAG	TTGCTCTTATGAAGCAGTGA
AVRDC-Okra17	ACGAGAGTGAAGTGGAACTG	CTCCTCTTTCCTTTTTCCAT
AVRDCOkra21	TCATGTCTTTCCACTCAACA	CCAAACAAAATATGCCTCTC
AVRDC-Okra28	CCTCTTCATCCATCTTTTCA	GGAAGATGCTGTGAAGGTA
AVRDC-Okra39	TGAGGTGATGATGTGAGAGA	TTGTAGATGAGGTTTGAACG

Source: **Schafleitner et.al.,2013**

Steps to undergo for protein profilling:

- Fruit samples of all parents and hybrids.
- Utilization of SDS- PAGE method for protein banding examination as described by Laemmli (1970).

4) Statistical analysis:

- Estimation of heterosis (Gardner and Eberhart, 1966; Gardner, 1967)
- Analysis of gene action (Hill 1982, Lynch 1991)
- Genetic diversity (by UPGMA method, GenALEx 6.3 software)

5) Marphological data:

Table No.4

Serial No.	Traits
1	Days to 50% Emergency
2	Days to first flowering
3	Days to 50 % flowering
4	Days to first Pod Formation
5	Days to maturity
6	Plant height
7	Stem diameter
8	No. of branch
9	Fruit length (cm)
10	Fruit weight
11	Fruit yield
12	Pod/plant

EXPECTED OUTCOMES

At completion of the work it is expected to observe adequate magnitude of heterosis by some of F1 hybrids among the 66 cross combination for various quantitative or qualitative traits. The purpose of present study is to obtain parent genotypes with good GCA and SCA for desired characters through the possible cross combination. The motive of this experimentation is to provide with useful information and data regarding heterosis analysis for further crop improvement programmes.

REFERENCES

1) Adiger, S., Shanthakumar, G. and Salimath, P.M. 2013. Selection of parents based on combining ability studies in okra (Ablemoschus esculentus (L.) Moench). *Karnataka J. Agric. Sci.*, 26(1): 6-9.
2) Akinyele, B.O., Taye, T. (2007) Effect of Variation in Soil Texture on the Vegetative and Pod Characteristics of Okra (Abelmoschus esculentus (L.) Moench. *International Journal of Agricultural Research*, 2(2): 165-169
3) Castillo, I. J. B., Angelia, M. R. N., Torio, M. A. O., & Belina-Aldemita, M. D. (2017). Antihypertensive property of the peptic and chymotryptic hydrolysates derived from the crude protein extract of okra [Abelmoschus esculentus (L.) Moench] seeds. *International Food Research Journal*, *24*(6), 2586-2592.
4) Dhruve, J. J., Shukla, Y. M., Shah, R., Patel, J., & Talati, J. G. (2015). CONTRIBUTION OF OKRA (ABELMOSCHUS ESCULENTUS L.) SEEDS TOWARDS THE NUTRITIONAL CHARACTERIZATION. *World J. Pharmacy and Pharmaceutical Sciences*, *7*, 1009.
5) Dutta P.C. and Naug A (1968) A few strains of *Abelmoschus esculentus* (L.) Moench their karyological in relation to phylogeny and organ development. Beitr. Biol. Pflanzen. 45: 113-126.

6) Helaly, A. A., Shalan, M. A., Abdou, A. A., & Alabd, M. T. Molecular Identification of Nine Okra Landraces by SDS-PAGE and RAPD Markers technique.
7) Kelly, J. D. (1995). Use of random amplified polymorphic DNA markers in breeding for major gene resistance to plant pathogens. *HortScience*, *30*(3), 461-465.
8) Khan, A. A., & Azmat, M. A. (2013). Assessment of genetic diversity in okra (Abelmoschus esculentus L.) using RAPD markers. *Pakistan Journal of Agricultural Sciences*, *50*(4).
9) Kumar, N. (2006). *Breeding of Horticultural crops*. New Delhi: New India Publishing Agency, pp 173–7.
10) Kumar, N. 2014. Heterosis and combining ability for yield and yield attributes in okra (Abelmoschus esculentus (L.) Moench). M. Sc., Thesis, Navsari Agricultural University, Navsari.
11) Kumar, P. S., & Sreeparvathy, S. (2010). Research Article Studies on Heterosis in Okra (Abelmoschus esculentus (L.) Moench). *Electronic Journal of Plant Breeding*, *1*(6), 1431-1433.
12) Kumar, P. S.; Sriram, P. and Karuppiah, P. (2006). Studies on combining ability in okra (*Abelmoschus esculentus* (L).Moench). *Indian J. Hort*. 63 : 182-184.
13) Kumar, S., & Reddy, M. T. (2016). Combining ability of inbred lines and half-diallel crosses for economic traits in okra (Abelmoschus esculentus (L.) Moench). *Jordan J. Agr. Sci*, *12*(2), 479-498.
14) Kumar, S., Singh, A. K., & Das, R. (2015). Combining Ability and Gene Action in Relation to Pod Yield of Okra [Abelmoschus esculentus (L.) Moench]. *Environment & Ecology*, *33*(3), 1155-1162.
15) Lamont, W. (1999) Okra a versatile vegetable crop. *Hort. Technol*, 9: 179-184.
16) Leonardi, A., Damerval, C., Hebert, Y., Gallais, A., & De Vienne, D. (1991). Association of protein amount polymorphism (PAP) among maize lines with performances of their hybrids. *Theoretical and applied genetics*, *82*(5), 552-560.
17) Lyngdoh, Y.A., Mulge, R. and Shadap, A. (2013). Heterosis and combining ability studies in near homozygous lines of okra [Abelmoschus esculentus (L.) Moench] for growth parameters. *The Bioscan* 8(4):1275–279.

18) Maciel, G. M., Luz, J. M., Campos, S. F., Finzi, R. R., & Azevedo, B. N. (2017). Heterosis in okra hybrids obtained by hybridization of two methods: traditional and experimental. *Horticultura Brasileira*, *35*(1), 119-123.

19) Mehta, N., Asati, B. S., & Mamidwar, S. R. (2008). Heterosis and gene action in okra. *Bangladesh Journal of Agricultural Research*, *32*(3), 421-432.

20) Moser, H., & Lee, M. (1994). RFLP variation and genealogical distance, multivariate distance, heterosis, and genetic variance in oats. *Theoretical and Applied Genetics*, *87*(8), 947-956.

21) Nagesh, G.C., Mulge, R., Rathod, V., Basavaraj, L.B. and Mahaveer, S.M. (2014). Heterosis and combining ability studies in okra [Abelmoschus esculentus (L.) Moench] for yield and quality parameters. *The Bioscan* 9(4):1717–23.

22) Osawaru, M. E., Ogwu, M. C., Chime, A. O., & Osifo, E. (2012). Morphological characterization of fruits and protein profiling of nine accessions of cultivated Okra species in Nigeria. *Biological and Environmental Sciences Journal for the Tropics*, *9*(3), 794-807.

23) Patel, B. G., & Patel, A. I. (2016). Heterosis studies in okra (Abelmoschus esculentus (L.) Moench). *Annals of Agricultural and Environmental Sciences*, *1*(01).

24) Patel, R. K. (2015). Heterosis for green fruit yield and its contributing traits in okra (Abelmoschus esculentus (L.) moench). *BIOINFOLET-A Quarterly Journal of Life Sciences*, *12*(1a), 60-63.

25) Raghuvanshi, M., Singh, T.B., Singh, A.P., Singh, U., Singh, V.P. and Singh, B. 2011. Combining ability analysis in okra (Abelmoschus esculentus (L.) Moench). *Veg. Sci*., 38(1): 26- 29

26) Rewale, V.S., Bendale, V.W., Bhave, S.G., Madav, R.R. and Jadhav, B.B. (2003a). Heterosis for yield and yield components in okra. *J Maharashtra Agric Univ* 28(3):247–9.

27) Saifullah M, Rabbani MG, Garvey EJ (2010). Estimation of genetic diversity of okra (Abelmoschus esculentus L. Moench.) using RAPD markers genotypes. *SAARC J. Agric.* 8:19-28

28) Schafleitner, R., Kumar, S., Lin, C. Y., Hegde, S. G., & Ebert, A. (2013). The okra (Abelmoschus esculentus) transcriptome as a source for gene sequence information and molecular markers for diversity analysis. *Gene*, *517*(1), 27-36.

29) Smith, O. S., Smith, J. S. C., Bowen, S. L., Tenborg, R. A., & Wall, S. J. (1990). Similarities among a group of elite maize inbreds as measured by pedigree, F 1 grain yield, grain yield, heterosis, and RFLPs. *Theoretical and Applied Genetics*, *80*(6), 833-840.

30) Song, K. M., Suzuki, J. Y., Slocum, M. K., Williams, P. M., & Osborn, T. C. (1991). A linkage map ofBrassica rapa (syn. campestris) based on restriction fragment length polymorphism loci. *Theoretical and Applied Genetics*, *82*(3), 296-304.

31) Tindall, H.D. (1983) Vegetables in the tropics. u: Macmillan Education Limited, London: AVI.

32) Vasconcelos, M. J., De Barros, E. G., Moreira, M. A., & Vieira, C. (1996). Genetic diversity of the common bean Phaseolus vulgaris L. determined by DNA-based molecular markers. *Embrapa Milho e Sorgo-Artigo em periódico indexado (ALICE)*.

33) Wamm, D. T., Kadams, A. M., & Jonah, P. M. (2010). Combining ability analysis and heterosis in a diallel cross of okra (Abelmoschus esculentus L. Moench). *African Journal of Agricultural Research*, *5*(16), 2108-2115.

34) Wang ZY, Second G, Tanksley SD (1992) Polymorphism and phylogenetic relationships among species in the genus Oryza as determined by analysis of nuclear RFLPs. *Theoretical and Applied Genetics* 83: 565–581

35) Woolfe, M. L., Chaplin, M. F., & Otchere, G. (1977). Studies on the mucilages extracted from okra fruits (Hibiscus esculentus L.) and baobab leaves (Adansonia digitata L.). *Journal of the Science of Food and Agriculture*, *28*(6), 519-529.

36) Yıldız, M., Koçak, M., & Baloch, F. S. (2015). Genetic bottlenecks in Turkish okra germplasm and utility of iPBS retrotransposon markers for genetic diversity assessment. *Genetics and Molecular Research*, *14*(3), 10588-10602.

37) Yuan, C. Y., Zhang, C., Wang, P., Hu, S., Chang, H. P., Xiao, W. J., ... & Guo, X. H. (2014). Genetic diversity analysis of okra (Abelmoschus esculentus L.) by inter-simple sequence repeat (ISSR) markers. *Genet. Mol. Res*, *13*(2), 3165-3175.