Genetic Variation Among and Within Okra

Abelmoschus esculentus L. Landraces in Jordan

By

Ibrahim Mohammad Rawashdeh –

Supervisor by

Prof. Mahmoud Kasrawi

Co-Supervisor

Dr. Sameer Masoud

Submitted in Partial Fulfillment of the Requirements for the

Degree of Master of Science in

Plant Production/Horticulture

Faculty of Graduate Studies

University of Jordan

August 1999

Committee Decision

This thesis was successfully defended and approved on August 4th, 1999.

Examination Committee	Signature
Dr. Mahmoud Kasrawi / Chairman Prof. of Plant Breeding	M.A. Kasrawi
Dr. Sameer Masoud / Co-Supervisor Assistant Prof. of Molecular Plant Pathology	Masoud's
Dr. Naim Sharaf Prof. of Entomology	N. Sharaf
Dr. Fadhil M. Al-Mohammadi Prof. of Vegetable Physiology	Fadhil M. Al-Mohammadi
Dr. Nazir Al-Hadidi Associate Prof. of Vegetable Crops	N. Hadidi

DEDICATION

To My.....

Father .....

Mother .....

Brothers .....

Sisters .....

ACKNOWLEDGEMENT

Field work carried out in the Experimental Station at the University of Jordan Campus / Amman during 1998 and the Laboratory work was carried out at the DNA Laboratory at NCARTT.

My deepest gratitude and appreciating goes to my advisor Prof. Mahmoud Kasrawi and Co-advisor Dr. Sameer Masoud for their patience, constant encouragement, moral support, their continuos supervision advice through out the course of research work , also my thanks express to the other examination committee members Prof. Naim Sharaf, Prof. Fadhil M. Al-Mohammadi and Dr. Nazir Al-Hadidi.

I would like to express my thanks to NCARTT and the Germany developing agency (GTZ) for funding my study. My thanks are also extended to my brothers Mahmoud and Ahmad Rawashdeh, Dr. Salwa Al-Bodour, Dr. Kamal Khair Allah, Agri. Engineer Sahim Al-Tawarh, Siham Al-lozi, Hussein Migdadi, Fadil Ismail, Maha Syouf, Miss Waff's Qubaj and Shadwan Al-Masri for her continous support also to Khalid Al-Absi and Muhanned for their help each in his own way.

At last but not least, my special thanks to my parents, brothers, sisters for their continuous helping and support.

LIST OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF APPENDICES

Abstract

Genetic Variation Among and Within Okra *Abelmoschus esculentus* L. Landraces in Jordan

By

Ibrahim Mohammad Rawashdeh

Supervisor by

Prof. Mahmoud Kasrawi

Co-supervisor

Dr. Sameer Masoud

Genetic variation among and within nineteen okra landraces in addition to one cultivar was estimated based on morphological differences between genotypes in the field. Five landraces, and one cultivar were further analysed to detect variations among and within landraces at the DNA level using Random Amplified Polymorphic DNA (RAPD) technique. In addition different horticultural and fruit traits were studied such as days to flowering, plant height at flowering, final plant height, immature pod width, immature pod length, mature pod length, immature pod weight, pod per plant, pod yield per plant, seed per pod, stem color, leaf color, leaf petiole color and pod color.

Statistical analysis using mean, correlation, standard deviation and coefficient of variability, indicated significant variability either among or within some landraces. The highest morphological variation registered was for the number of pods per plant (50.2%) and the lowest one was for days to flowering (8.3%) this was indicative of the

effectiveness of selection also, wide range of variability for most quantitative genetic traits.

Some okra landraces shared the same color in stems pods and leaves, indicating that common genes controlling these traits. Days to flowering, pod yield per plant, immature pod width and pod color could be used to discriminate between genotypes.

Data obtained from arbitrary decamer-oligonucleotide primers have indicated that the RAPD technique could be useful to detect variation among and within landraces. Primer OPA13 were particularly efficient to amplify banding patterns among and within okra landraces. One landrace could be distinguished with caution from other landraces using the presence or absence of a DNA fragment size of 386 bp or 1000 bp when this primer was used.

RAPD analysis could be used efficiently for detecting variations among and within landraces and cultivar. Although okra landraces were different morphologically, but at the DNA level variations were not enough to obtain a characteristic DNA polymorphism for each landrace or cultivar. Analysis of additional arbitrary primers in RAPD revealed that different primers could be used in breeding programs.

This study initiated molecular characterization for identification and collecting okra of different genetic resources. It lays foundation for breeding programs.

Introduction.

Okra (*Abelmoschus esculentus L.*), an annual herbaceous plant belonging to malvaceae, is one of the important vegetable crops grown in Jordan. In 1995, the total area planted to okra was 7820.7 dunum produced 2684.7 tons. However, in 1996, the total area decreased to 6868.0 dunum and the production to 2094.5 tons. In 1997, the area planted with okra was increased to 10111.7 dunum with a production of 2841.9 ton (Department of General Statistic, 1995-1997). So, even though the productivity per area were decreased in this periods because shortage in rain full.

Most of okra cultivars planted in Jordan are introduced from abroad such as "Clemson Spineless", but some growers still use landraces. These landraces are valuable source of genetic variation and may possess some interesting characteristics such as red color of fruit and adaptation to prevailing local environmental conditions. Okra landraces are becoming extinct from all parts of Jordan, and very rapidly replaced by imported cultivars.

Landraces of okra collected in 1995/1996 from different areas of Jordan by National Center of Agricultural Research and Technology Transfer (NCARTT) were stored in the genetic resource unit in NCARTT (personal communication). These landraces have not been genetically evaluated for the most important traits.

Polymerase Chain Reaction (PCR) has become a basic and essential tool for detecting genetic variation and elucidate unknown DNA sequences (Newton and Graham, 1995). Recently, DNA based molecular techniques have increasingly been used to detect genetic variation among and within cultivars and landraces (Franham, 1996). Among these, the Random Amplified Polymorphic DNA (RAPD) technique could amplify polymorphic regions within the genome defined by the primer sequence. RAPD is based on PCR technique and is intensively used to study the genetic variation in different plant population without previous sequence information (Vazquez *et al.*1996). RAPD is simple, fast and reveals sequence variation in form of bands to discriminate species and cultivars.

The objectives of the present work were to study the genetic variability of several morphological and horticultural traits among and within Jordanian okra landraces in the field and to examine the genetic variation of these landraces at the DNA level using RAPD technique.

2- Literature Review.

2-1 Genetic Variation.

Landrace or primitive cultivar is a mixed population possessing large hereditary types (Kuckuck *et al.*,1991) and considered as a source of genetic diversity for successful development of improved cultivars (Frankel, 1977). Ariyo (1990) reported that the genetic variability is one of the fundamentals of plant breeding which is a major tool being used to compensate with ever-increasing pressure of expanding world population on food production.

In okra, genetic variations were observed in 1806 accessions that belong to eight species, with maximum variability in *Abelmoschus esculentus* (Rana and Thomas, 1990). Wide range of genetic diversity existed among and within okra accessions were collected in Sudan (El – Tahir, 1990). Typical varieties of okra which constitute a large relatively homogenous collection showed small variations among country groups (USA, Turkey, Syria, China) and large variations in those collected from producing countries (Martin, 1981).

2-2 Morphological Characterization.

2-2-1 Flowering.

Days to flowering in okra has been classified by International Board for Plant Genetic Resources (IBPGR) as a number of days from sowing to the first open flower (Charrier, 1984). Kaul *et al.* (1978) found that the time taken from sowing to first pod harvest in okra varied from 54 to 80 days. On the other hand, the best time to observe the growth of *Abelmoscus esculentus* is around 80 to 90 days after sowing (Hamson *et al.*, 1990). Days to flowering is a character used in studying variability among okra genotypes (Ariyo and Aken' Ova, 1986) and remarkable variation exists in days to flowering of okra accessions (Bisht *et al.*, 1997). Number of days to flowering had a large direct effect on earliness and pod yield (Raji, 1994).

Chheda and Fatokun (1990) reported that the height of okra plants at flowering varied from 26-112 cm in okra accessions. However, genotypic coefficient of variation was the highest in plant height at flowering, indicating the effectiveness of selection (Ariyo, 1990). Helmy (1991) found high heterogeneity of plant height at flowering using coefficient of variation. Plant height at flowering and maximum number of nodes are important traits in selection for high yield (Singh and Singh, 1979).

2-2-2 Pod Width and Length.

Pod width in okra showed a wide variation and ranged from 2 to 4 cm (Chheda and Fatokun, 1990) and it is used to distinguish between okra genotypes (Singh *et al.*, 1977). Ariyo (1987) found that edible pod width is correlated significantly and positively with pod yield and influences directly or indirectly to the pod yield. Guirgis *et al.* (1990) suggested that the pod width is an important factor in breeding programs for yield improvement. The continuous variation of okra pod width in few classes indicated that immature fruit diameter is quantitatively inherited with probably few genes involved in controlling this trait (Salameh, 1998).

Fruit length has been classified by the International Board for Plant Genetic Resources (IBPGR) into three classes: less than 7 cm, 8 –15 cm, more than 15 cm (Charrier, 1984). Fruit length of okra germplasm exhibited a wide variation range of 6 – 18 cm (Chheda and Fatokun, 1990). Velayudan and Upadhaya (1994) found variation in fruit length of okra accessions collected from Nepal. Positive and significant correlations (0.776) between fruit length and pod yield per plant were found in some populations collected from Egypt (Guirgis *et al.*, 1990). Pod length is genetically controlled, which did not change irrespective of the environment (Raji, 1994).

Continuos variation in fruit length of F_2 population generated from landraces in Jordan indicated that this trait is a quantitative one and is controlled by a few genes (Salameh, 1998).

2-2-3 Yield and Yield Components.

The total seed yield produced is based on number of pods per plant and seed per pod (Martin *et al.*, 1981). Number of seed per fruit ranged from 68 to 97 with variation in size and color for certain parents (Fatokun, 1987) and from 50 to 116 with an average of 77 for 47 okra accessions (Kaul *et al.*, 1978). Kaul *et al.* (1978) and Ariyo (1990) found that seed yield per plant and number of pods per plant are highly correlated with pod yield and appeared to be influenced by environment. Number of fruits per plant in okra accessions ranged from 3 to 18 with an average of 6.5 to 14.8 (Chheda and Fatokun, 1990).

Okra pods are sold on the basis of number instead of weight in Nigeria and African markets (Raji, 1994). Ariyo (1987) indicated that the edible pod yield per plant and days to flowering accounted 57.83% of total genetic variance indicating that the two characters would be reliable in distinguishing among okra varieties. March and Jones (1990) recorded total number of fruits harvested per plant was two or more for each day. While Singh and Singh (1979) demonstrated the first fruiting node at short distance will be helpful in increasing the number of fruits per plant. Pod

yield was influenced mainly by the number of pods per plant (Kaul *et al.*, 1978). Also Singh and Singh (1979) reported that yield of pods had a positive and significant association with the number of pods per plant. Dewdar *et al.* (1987) reported that pod yield per plant was based on number of pods per plant and weight in gram per plant.

2-2-4 Pod Color.

Pod color in okra has been classified by IBPGR into four classes: yellowish green, red patches, green and red (Charrier, 1984). Diversity in fruit color was observed among and within collected okra accessions from Sudan including green, green with red, yellowish green and red (El – Tahir, 1990). Hamon *et al.* (1990) reported that fruit color and size are the most important variabilities, or at least visible ones in okra germplasm. However, Hamon (1990) indicated that color in okra fruits is not easy to assess for homogeneous accessions due to uncertainty with color variations and to superimposition of one color over other. Therefore, it is rare to find a totally homogenous accession for fruit color due to complex genetic base of fruit pigmentation. Variations in fruit color and other characters for certain accessions collected from Nepal were observed by Velayudan and Upadhyay (1994). On the other hand, Martin (1981) indicated that the anthocyanin (red color) in okra tends to be produced in various organs at

the same time. Salameh (1998) showed homozygous okra fruit color and red color is dominant and controlled by two independent genes.

Kalia and Padda (1962) reported that fruit color of okra character is governed by multiple allelic series in which green and cream fruit color are allelic to each other, also they reported that purple fruit color was dominant over green and cream one.

2-2-5 Stem Color.

Stem color was classified by IBPGR into fourth classes: 1 to green, 2 to green with red patches and 3 to purple and 4 to red (Charrier, 1984). Hindagala *et al.* (1990) reported that the predominant stem color was green, but a few accessions were reddish or purple. Red pigmentation of the main stem, branches and petioles is apparently controlled by two genes (Erickson, 1962). Salameh (1998) also reported that red stem color in a population developed from landraces of Jordan, is dominant and controlled by two genes.

2-2-6 Final Plant Height.

Final plant height with a smaller internodal length is an important factor in increasing okra yield, okra pods borne on every node of the plant (Sharma and Mahajan, 1978). Mishra and Chhonkar (1979) reported that final plant height had a wide range of variation, also showed higher genotypic variance (173.63) and hertability (19.65%). Final plant height

was positively correlated with pod length and net yield (Gurigis *et al.*, 1996). Raoswamy and Sathyavathi (1977) found that genotypic and phenotypic coefficient of variation were less for final plant height and low heritability in the F_2 generation, indicating much addition portion of the variation lost in F_2 generation.

2-3 Molecular Characterization.

2-3-1 Polymerase Chain Reaction (PCR) Application.

Genetic variations and diversity has been previously based mainly on the gene products such as morphological traits and proteins. Recently ,a new technique, Polymerase Chain Reaction (PCR), has attracted interest for identification and evaluation of plant material (Moreno, 1995), because it directly analyze the genetic make up of an organism.

PCR was invented in 1985 and it is a technique amplify DNA fragment to billion copies without needing living tissues (Newton and Graham, 1994). The major advancement that made PCR key procedure is the introduction of a new enzyme from the bacteria *Thermus aquaticus* (*Taq* polymerase) (Newton and Graham, 1994). The *Taq* polymerase simplified the procedure, improved the specificity yield and length of products amplified by PCR (Saiki *et al.*, 1988). PCR detects rapidly the genetic variation in a few hours instead of days or months (Williams and Roland, 1994).

The PCR technique is characterized by it's high speed, selectivity, sensitivity and require a small amount of DNA (Weising *et al.*, 1994). The *in vitro* amplification of DNA require the following steps of temperature cycles; denaturation, separate the double strands of DNA at temperature around 94°C, annealing, which enable the primer to anneal with a single strand of DNA at a specified temperature 33°C - 65°C, and extension which is DNA synthesis by a thermostable DNA polymerase at temperature 70-75°C (Weising *et al.*, 1994). The purity and yield of reaction products are dependent on several critical parameters such as annealing temperature (Rychlik *et al.*, 1990).

2-3-2 Randomly Amplified Polymorphic DNA (RAPD).

Randomly Amplified Polymorphic DNA (RPAD) technique is an efficient approach for detection of DNA polymorphism, which allowing the analysis of a single seed or young seedlings (Gregor *et al.*, 1994). In this technique, genomic DNA is amplified by using arbitrary primers without requiring prior sequence information (Vazquez *et al.*, 1996). The amplified DNA fragments are detected by the agarose gel electrophoresis. DNA pattern could be viewed directly by UV light after ethidium bromide staining (Gregor *et al.*, 1994 ; Sambrook *et al.*, 1989).

The RAPD technique has been successfully used to identify certain cultivars, study the genetic diversity, distinguish between species, maintain

cultivar purity and detect the genetic variation (Tivang *et al.*, 1996; Degani *et al.*, 1998; Moreno *et al.*, 1995; Golembiewski *et al.*, 1997).

RAPD markers have been used in several plants with different application to distinguish between lentil landraces (Ferguson *et al.*, 1998), pistachio cultivars (Dollo *et al.*, 1995), and Heliconia (Kumar *et al.*, 1998). Discrimination between tomato cultivars to generate a polymorphic DNA marker was also used based on combination of two primers in a single PCR (Klein – Lankhors *et al.*, 1991; Foolad *et al.*, 1993). In addition, RAPD was used in combination with morphological traits for identification and discrimination between potato cultivars (Sosinski and Douches, 1996). Also, RAPD was used to distinguish, evaluate the collected plants or landraces, emphasized the similarity or dissimilarity between and within landraces or cultivars of eggplant (Kariahloo *et al.*, 1995), and of pepper cultivars (Vazquez *et al.*, 1996), and in collard landraces (Farnham, 1996).

3- Materials and Methods.

3-1 Morphological Characterization of Okra Landraces.

3-1-1 Plant Materials and Cultural Practices.

Seed of nineteen landraces of okra were obtained from the Genetic Resource Unit at NCARTT. These landraces were collected from different regions Jordan in 1995/1996 (Table 1). One commercial cultivar "Clemson Spineless" was used as a control. Seed of landraces were soaked for 24 hours in tap water, then were sown in the seedling trays on March 31,1998. On April 22,1998, seedlings of 23-days-old were transplanted to open field in the Experimental Station at the University of Jordan Campus / Amman. The experiment layout was a randomized complete block design (RCBD) with three replications. Each plot consisted of a single 5 m long row with 20 plants per plot and with a spacing of 50 cm between rows and 25 cm between plants within a row. Black plastic mulch and drip irrigation system with drippers placed along the laterals at 20 cm were used. Irrigation was supplied at the time of planting and when need. Water soluble fertilizer (Amcolon) (20: 20 :20 NPK) at rate of 10 g/ plant was added after 40 days from sowing drench for each plant.

Soil borne pathogens were controlled after planting by soil drenching with "Tachigaren, a.i. Hemixazole 41.52%." at a rate of 20 ml /20L on April 29, 1998. Protection against aphids and whiteflies was made by spraying "Dursban, a.i.Chlopyrifos 40.8%" at a rate of 40 ml/20L and

"Vetaron, a.i. Methamidophos 60%" at a rate of 20 ml/20L on May 19, 1998, respectively. Plants were also protected against foliar fungal diseases like powdery mildew by spraying "Afugan, a.i. Pyrazophos EC 30%" at a rate of 8 ml/20L on May 4, 1998. Hand weeding between and within rows were done and continued throughout the growing season.

3-1-2 Data Collection.

Data were obtained from individual plants in each plot during the growing season. The following characters were recorded following IBPGR descriptor (Charrier, 1984).

1- Days to flowering: number of days from sowing the seed to the first opening of the flower were record daily.
2- Plant height: measured at the time of first flowering (of each landrace).
3- Pod width: measured when the fruits reached the marketing stage (3-5 days after flowering) by a Vernier caliper.
4- Pod length: measured when the fruits reached the marketing stage (3-5 days after flowering).
5- Pod color: observed visually when the fruits reached the marketing stage (3-5 days after flowering).
6- Stem color: observed visually after 42 days from sowing the seed.
7- Leaf color: observed visually after 42 days from sowing the seed.
8- Petiole color: observed visually after 42 days from sowing the seed.
9- Yield per plant: measured when pods reached the marketing stage (3-5 days after flowering).
10- Mature pod length: measured when pod turned into yellow color.

11- Number of seed per pod: counted after pods turned into yellow color and capsules were opened.

12- Number of pods per plant: measured at the stage of marketing and continued until the growth of the plant was stopped (defolation all leaves from plant).

13- Final plant height: measured when plants stopped growth.

Scoring of each character followed IBPGR descriptor (Charrier, 1984) and shown in Table 2.

Table (1): Okra landraces collected from different regions in Jordan (1995).

Landraces number	Province	Location
Jo 76	Irbid	Fou'ara –Hawar Junction
Jo 79	Irbid	Kora ,Al-Hammar area Tabaket fahel
Jo 80	Irbid	Kora,west dairabi-said
Jo 84	Irbid	Al Sakaia , 0.5 km from Kuifur asad
Jo 88	Irbid	Al Sakaia , 0.5 km from Kuifur asad
Jo 89	Irbid	Al Sakaia , 0.5 km from Kuifur asad
Jo 90	Irbid	Hawar
Jo 144	Irbid	Hakama
Jo 147	Irbid	Fou'ara
Jo 148	Irbid	Sama al Rousan
Jo 149	Irbid	Al Barha
Jo 150	Mafraq	Saba Al-Sir
Jo 151	Irbid	Sama
Jo 152	Irbid	Sama
Jo 153	Jarash	Rashadieh
Jo 154	Madaba	Ghernatah
Jo 155	Madaba	Mansourah
Jo 156	Irbid	Hussen Camp
Jo 157	Irbid	Barha
Jo 158	Irbid	Hussen Camp
(Clemson Spineless) hybrid cultivar.		

Source: (NACRTT) documentation.

Table (2): Score measurements for different characteristics of Jordanian okra landraces.

Character	Score
1- Stem color	1 Green
	2 Green with red patches
	3 Purple
	4 Red
2- Leaf color	1 Green
	2 Green with red viens
	3 Red
3- Fruit color	1 Yellowish green
	2 Green
	3 Green with red patches
	4 Red
4- Fruit length at maturity	1 Less than 7 cm
	2 From 8 to 15 cm
	3 More than 15 cm

Source: IBPGR (Charrier, 1984)

3-2-9 Statistical and Genetic Analysis.

Frequency distributions, means, standard deviations, coefficient of variations and correlations between pairs of the traits for landraces and control were calculated according to formulas outlined by Steel and Torrie, (1980).

$$\text{Mean} = \frac{\sum x}{n}$$

$$\text{Standard deviation} = \frac{\sum (x - \bar{x})^2}{n-1}$$

$$\text{Coefficient of variability} = 100 \times \frac{s}{\bar{x}}$$

$$\text{Correlation coefficient} = \frac{\sum (x - \bar{x})(y - \bar{y})}{(\sum (x - \bar{x})^2 \sum (y - \bar{y})^2)^{1/2}}$$

3-2 Molecular Characterization of Okra Landraces.

The molecular analysis protocols were done in the DNA laboratory at NCARTT. Tools (Appendix A) and solutions used during DNA extraction and PCR amplification were autoclaved to reduce the possibility of cross contamination and inconsistency in the amplification reactions. Safety procedures were followed by wearing laboratory coat, mouth mask, latix gloves and face sheild when UV light is used.

The Random Amplified Polymorphic DNA (RAPD) technique was used to detect the genetic variation among and within five okra landraces and control according to Karihaloo *et al.* (1995) method with some modifications include concentrations.

3-2-1 Plant Material.

Five okra landraces that showed morphologically significant differences based on earlier field evaluation and one commercial cultivar "Clemson Spineless" were chosen for RAPD analysis. Four different plants for each landrace and the commercial cultivar were selected randomly and used in the molecular analysis of variation. True leaves from two weeks-old seedlings were collected in aluminum foil bags and transferred in ice box into the laboratory for analysis.

3-2-2 DNA Isolation from Leaves.

DNA extraction was performed according to Doyle and Doyle (1990) with minor modifications include concentrations. Leaves were rinsed two times in sterile distilled water, then blotted between two filter papers for 10 minutes. One hundred milligrams of a newly growing tissue were placed in 1.5 ml microfuge tubes and grounded by a screw driver in the presence of liquid nitrogen.

DNA extraction buffer [2 X CTAB (2% hexadecyl trimethyl ammonuim bromide), 1.4M NaCl, 100mM Tris-HCl, 20mM EDTA

(ethylenediaminetetraacetic acid), 1% 2-mercaptoethanol, 1% polyvinyl-polypyrrolidone], were diluted into 1.33 X CTAB using distilled water. After addition of 450µl of 1.33 X CTAB DNA extraction buffer to the ground tissues, samples were incubated at 65°C for 15-30 minute in water bath mixed occasionally mixing. Chloroform/isoamyl alcohol (24:1; v:v) [(600 µL)] was added to each tube and vortexed for few seconds then centrifuged at 14000 rpm [Eppendorf-5415C] for four minutes. The aqueous phase (approximately 200 µL) was transferred into a new tubes which contains l µL of 10mg/ml RNase [see Appendix B-II] and incubated for 5-10 minutes at 37°C. Chloroform/ isoamyl alcohol extraction was repeated and the top phases were placed in new sterile tubes. Nucleic acids were then precipitated by the addition of 120 µL isopropanol. Contents were mixed and centrifuged for five minutes at 14000 rpm [Eppendorf-5415C]. Alcohol was poured out and 1ml of 70% ethanol was added for tube and each stored at -20°C. Next day, ethanol was poured out and the tubes left for air drying. Pellets were dissolved in 50 µL TE buffer [Appendix B-2] at 65°C for 5 minutes and stored at -20°C until template DNA's were characterized and used in PCR reactions.

3-2-3 Measurement of DNA Yield.

Preliminary tests of quantify and to determine the quality of the isolated DNA have been conducted by loading 5µl of DNA solution and 2µl of gel loading buffer (see Appendix B-9) into 0.7% agarose gel electrophoresis and separated for 45 minutes.

Yield of DNA was also measured by fluoremetry (DYNA quant 200), using 10 X TNE buffer [Appendix B-4] containing Hoechst dye (Pharmacia; Sweden). Isolated DNA (2 µl) and (2ml) of 10 X TNE buffer were used to estimate the concentration of DNA (Table 4).

Table (3): DNA concentration of five okra landraces collected from different regions in Jordan and the commercial cultivar "Clemson Spineless".

Landraces	Plant number	Concentration (ng/μl)
Jo 76	1	17
	2	15
	3	18
	4	13
Jo 84	1	12
	2	15
	3	15
	4	13
Jo 148	1	16
	2	31
	3	16
	4	19
Jo 154	1	28
	2	15
	3	12
	4	19
Jo 156	1	17
	2	21
	3	19
	4	15
Control (Clemson Spineless)	1	19
	2	12
	3	16
	4	28

Table (4): Primers used in RAPD to identify the five Jordanian okra landraces and the commercial cultivar "Clemson Spineless".

Series	Primer Code	Sequence 5′ to 3′
1.	OPA02	TGCCGAGCTG
2.	OPA04	AATCGGGCTG
3.	OPA06	GGTCCCTGAC
4.	OPA07	GAAACGGGTG
5.	OPA08	GTGACGTAGG
6.	OPA09	GGGTAACGCC
7.	OPA10	GTGATCGCAG
8.	OPA12	TCGGCGATAG
9.	OPA13	CAGCACCCAC
10.	OPA15	TTCCGAACCC
11.	OPA16	AGCCAGCGAA
12.	OPA18	AGGTGACCGT
13.	OPA19	CAAACGTCGG
14.	OPB05	TCGGCCCTTC
15.	OPB03	CATCCCCCTG
16.	OPB06	TGCTCTGCCC
17.	OPB14	TCCGCTCTGG
18.	OPM05	CCCAACGTGT
19.	OPM13	GGTGGTCAAG
20.	OPO02	ACGTAGCGTT

3-2-4 RAPD Reactions and PCR Program.

Different assays to optimize the template DNA concentrations were conducted over a range of 1µl to 10 µl of extracted DNA. Also different annealing temperatures (37°C, 35°C and 33°C) were experimented. Consistent banding patterns were obtained at a template DNA concentration of 5µl and at annealing temperature of 35°C.

The RAPD reactions were performed in 25 µL final volume containing 2.5 µL of 10 X reaction buffer [see Appendix B-3], 2.5 µL of 2-deoxyribonucleic acids [dNTPs; 2mM each of dATP, dTTP, dGTP and dCTP], 1 µL of each primer (one or combinations of two or three were used) (Table 4) (Operon Technologies INC., CA., USA), 0.2 µL *Taq* DNA polymerase (5units/1µL), 5µL of genomic DNA, and the remaining volume was completed with deionized water to the final volume of 25µL. *Taq* polymerases and 10 X buffers used were either from Sigma, Perkin Elmer or Promega (Appendix A-3) while dNTPs were used from Promega. The PCR program was set according to Karihaloo *et al.* (1995) with minor modifications. The samples were subjected to half minute at 94°C, followed by 40 cycles of 1 minute at 94°C as denaturation step, 1 minute at 35°C as annealing step and 2 minute at 72°C as extension step, and the final cycle was followed by 5 minute at 72°C. Following amplification, samples were

maintained at 4°C until electrophoresis. The RAPD reactions were performed in Gene Amp PCR system 9600, Perkin Elmer Ltd.

3-2-5 Gel Electrophoresis Preparation and Detection of Amplified DNA.

Amplified products were separated electrophoretically in 1.4% agarose and 0.5 X TBE buffer (see Appendix B-5-2). The agarose was dissolved in a microwave oven and left to cool down to (50-55)°C. Ethidium bromide (5 µL) [see Appendix B-10] was added to the agarose mixture before pouring into the gel tray. Electrophoresis casting tray were washed by ethanol and distilled water then air dried. Two ridges and comb were placed on tray, then agarose solution was poured to a height of 5 mm. After 20 minutes, comb were removed and 0.5 X TBE buffer was added to a level of around 0.5 cm above the gel. Amplified DNA (10 µL) with (2 µL) loading buffer (see Appendix B-9) for each sample were used. Both cathode and anode were connected with electrophoresis power supply (Phero-Stab 330) at (100 volts) for about (80) minutes and when bromophenol blue dye migrated to about 6-7.5 cm from the wells. DNA was visualized using ultraviolet light transilluminator of 254nm and photographed using MP- 4^+ Polaroid camera with black and white Polaroid film 667.

3-2-6 Molecular Weight Estimation of PCR Products.

Molecular weights (bp) of different DNA fragments of RAPD products were estimated using standard curves. The size of amplification products was determined using DNA PCR marker [50-2000 pb, Sigma, USA.] that was run on every agarose gel.

3-2-7 Analysis of RAPD and Marker Scoring.

Amplification products (bands) of five okra landraces and control "Clemson Spineless" were scored either present (+) or absent (-) and identified by sizes in base pair. Only amplification products that consistently appeared between runs were rated.

Similarity was calculated according to the formula suggested by Weising *et al.* (1995)

$$S = \frac{2\,n_{ab}}{n_a + n_b},$$

where n_a and n_b represent the total number of bands present in lanes a and b, respectively, n_{ab} is the number of bands which are shared by both lanes.

4- Results and Discussions.

4-1 Morphological Characterization of Okra Landraces.

The analysis of variance (Appendix C) revealed significant differences among nineteen okra landraces collected from different regions in Jordan for all traits, indicating the presence of appreciable genetic diversity among landraces. Similar results were observed in okra varieties (Long Green, Pusa Sawani and Kalianpur Bonia) which were selected as parents on the basis of their wide range in adaptability, geographical diversity and desirable agronomic character in Nigeria (Singh and Singh, 1979). Differences among individual plants in each landrace were found not significant.

4-1-1 Days to Flowering.

Number of days to flowering for individual plants of nineteen okra landraces and the cultivar "Clemson Spineless" ranged from 63 to 109 with an average of 86 days and C.V. of 8.3% (Table 5). The wide range for number of days to flowering and relatively intermediate value of C.V. might indicate the existence of variability among and within landraces which allows for possible selection for earliness. Significant differences in days to flowering were showed among landraces (Table 6). Average days to flowering ranged from 84 days in Jo 90 to 95 days in Jo 89. Although, means of days to flowering in all landraces were relatively higher

Table (5): Mean ($\bar{X}$), standard deviation (SD), range (R) and coefficient of variation (C.V.%) for morphological traits of okra landraces collected from different regions in Jordan and control "Clemson Spineless".

Characters	$\bar{X} \pm SD$	R	C.V.%
Days to flower	86 ± 8.1	63 - 109	8.3
Plant height at flowering (cm)	27 ± 11.4	10 - 100	37.3
Final plant height (cm)	58 ± 22.0	16-157	31.3
Immature Pod width (cm)	0.97 ± 0.1	0.67-1.9	10.5
Immature pod length (cm)	4.1 ± 0.7	2.5-5.9	16.3
Mature pod length (cm)	11.9 ± 2.9	3.0 - 20	20.9
Immature pod weight (g)	3.8 ± 1.0	1.5 - 10	23.5
Immature pods (no. / plant)	15 ± 7.8	2 - 58	50.2
Pods yield per plant (g)	56 ± 31.6	7 - 230	35.7
Seed per pod	61 ± 16.5	8 - 115	25.5

Total number of plants = 1200

Table (6): Range (R), mean ($\bar{X}$), standard deviation (SD) and coefficient of variation (C.V.%) for days to flowering, plant height at flowering and final plant height for nineteen okra landraces collected from different regions in Jordan and control grown in the Experimental Station at the University of Jordan Campus in 1998.

Landraces number	Days to flowering (days)			Plant height at flowering (cm)			Final plant height (cm)		
	Range	$\bar{X} \pm SD$	C.V.%	Range	$\bar{X} \pm SD$	C.V.%	Range	$\bar{X} \pm SD$	C.V.%
Jo 76	75-101	86 ± 5.8	6.7	10-52	28 ± 9.7	34.7	23-157	63±22.9	36.3
Jo 79	77-109	90 ± 8.8	9.8	16-39	23 ± 4.9	21.4	25-68	40±14.3	35.6
Jo 80	75-109	90 ± 9.4	10.4	13-75	29 ± 11.5	39.2	28-138	61±18.4	30.0
Jo 84	74-108	85 ± 5.5	6.4	12-38	19 ± 4.9	25.3	16-88	46±13.4	29.0
Jo 88	78-109	94 ± 6.9	7.4	15-60	36 ± 12.0	33.5	27-117	68±18.8	27.7
Jo 89	78-106	95 ± 8.2	8.7	15-82	33 ± 13.3	39.9	18-119	62±19.9	32.2
Jo 90	76-97	84 ± 5.6	6.7	12-40	21 ± 5.8	28.3	16-94	43±16.4	37.9
Jo144	78-95	87 ± 4.9	5.6	16-48	29 ± 8.6	29.3	23-103	57±14.0	24.7
Jo 147	77-109	92 ± 8.8	9.6	15-52	30 ± 11.2	37.7	28-127	60±17.2	28.5
Jo 148	67-109	86 ± 6.4	7.5	12-70	28 ± 7.8	28.2	19-151	73±22.5	30.8
Jo 149	78-109	89 ± 7.5	8.4	15-55	30 ± 10.6	35.9	28-108	65±18.5	28.5
Jo 150	77-107	98 ± 6.6	7.4	16-53	30 ± 8.4	28.4	34-94	60±17.0	28.2
Jo 151	63-109	89 ± 10.1	11.3	12-100	35 ± 17.1	44.6	23-138	70±28.8	41.2
Jo 152	75-102	85 ± 6.7	8.1	13-38	20 ± 4.9	24.4	26-79	48±10.6	22.1
Jo 154	74-109	86 ± 7.5	8.7	14-60	23 ± 9.4	40.3	23-102	50±17.5	34.7
Jo 155	76-100	85 ± 5.9	6.9	11-55	23 ± 8.1	35.5	23-107	54±17.9	33.4
Jo 156	76-108	90 ± 6.7	7.5	12-60	27 ± 8.9	33.2	22-101	52±12.9	24.8
Jo 157	78-109	89 ± 8.0	9.0	14-62	32 ± 12.0	37.2	37-127	73±18.7	25.6
Jo 158	76-108	88 ± 6.6	7.4	10-52	24 ± 9.4	38.9	16-112	55±18.3	32.4
*Control	70-96	82 ± 5. 2	6.4	14-27	18 ± 2.4	12.8	18-93	49±13.6	27.8
LSD at P ≤ 5%		3.88			6.07			18.58	

* = Clemson Spineless

Number of plants for each landrace = 60

than control cultivar, individual plants in some landraces flowered earlier than the control indicating that single plant selection for earliness within landraces could be possible. The number of days to flowering can be used as a criterion for selecting lines with short life span by selecting for fewer number of days to flowering. Early flowering cultivars will be best to areas with short growing season (Ariyo, 1987). Plants of Jo 151 and Jo148 distributed over a wide range (63-109), and (67-109), respectively (Table 6) indicating heterogeneity in days to flowering between plants for each landrace probably due to impurity of seed. Farmers collect seeds from the best plants without pollination control. Therefore, pollen contamination may occur since cross pollination of more than 50% is possible in okra. Some okra landraces behaved similarly in days to flowering such as Jo 84, Jo 152 and Jo 155; and Jo 157, Jo 148 and Jo 154. So, the result indicated that Jo 90 is the earliness in this traits.

Coefficient of variability was used to measure the variation for days to flowering among okra landraces. The highest C.V. (11.3%) was for Jo 151 and the lowest one (5.6%) was for Jo144. On the other hand, Jo 89, Jo 149, Jo 152 and Jo 154 showed the same values of C.V. and equal to the C.V. among the okra landraces. The relatively wide range for days to flowering (70-96) among plants of the control also indicate that environmental factors may affect this trait. It is easy to say that number of days to flowering controlled by genetic factors, whereas environmental

conditions affect it (Dewdar *et al.*, 1987). Days to flowering is a character used in study variability among okra genotypes (Ariyo and Aken'Ova, 1986) and remarkable variation exists in days to flowering of okra accessions (Bisht *et al.*, 1977).

4-1-2 Plant Height at Flowering.

Plant height at flowering among individual plants of nineteen okra landraces and the cultivar "Clemson Spineless" ranged from 10 cm to 100cm with an average of 27 cm and C.V. 37.3% (Table 5 P. 26). The very wide range for plant height at flowering and high value of C.V. may indicate the existence of variability among and within landraces which allow for possible selection for lower or intermediate plant height. Significant differences in plant height at flowering were showed among landraces (Table 6).

Average plant height at flowering ranged from 19cm in Jo 84 to 36 cm in Jo 88. Most landraces have means of plant height at flowering higher than control cultivar, but some individual plants in some landraces flowered at shorter plant heights, indicating that selection for short to intermediate plant height at flowering within landrace could be possible. Plants of medium height (77 cm) gave the highest yield and could be used for intercropping (Raji, 1994). Plants of Jo 151 distributed over a wide range (12-100 cm) with 44.6% C.V. indicated that heterogeneity for plant

height at flowering between plants for this landrace. The wide range in plant height at flowering were obtained in landraces Jo 76 and Jo 158 (10-52 cm) and Jo 84 and Jo 152 (12-38 cm). Plants of the control ranged in plant height at flowering from 14 to 27 cm indicating that this trait is affected by environment. On the other hand, the range was narrowest in the control cultivars suggesting that variation within landraces is not only due to environment but genetic variation may exist.

However, most of landraces displayed wide ranges in plant height at flowering with relatively noticeable coefficient of variation suggesting that this trait is quantitatively inherited.

Plant height at flowering revealed high heterogeneity through coefficient of variation (26%) measurements of original Eskandrani cultivar of okra in Egypt (Helmy, 1991). On the other hand, the relatively large genotypic coefficients of variation and heritability estimates recorded for height at flowering (Ariyo, 1990).

Correlation between paris of plants traits are important in plant breeding, because an improvement in one trait may result in desirable and /or undesirable changes in other traits, also correlation could be resulted from genetic causes. Plant height at flowering showed positive and significant correlation ($r = 0.637$) with days to flowering (Table 7) indicating strong associations among these trait. Therefore, selection for shorter stems at flowering is a strong indication of earliness. For example,

Table (7): Correlation's among different pairs of agronomic traits of okra landraces collected from different regions in Jordan and control "Clemson Spineless" grown in the Experimental Station at the University of Jordan Campus in 1998.

Characters	1	2	3	4	5	6	7	8	9	10
	Days to flowering (days)	Plant height at flowering (cm)	Immature pod weight (g)	Immature pod width (cm)	Number of pods per plant	Seed/pod	Final plant height (cm)	Immature pod length (cm)	Mature pod length (cm)	Pods yield/ plant (g)
1	1	0.637 **	0.056 *	0.97**	-0.194**	0.007	0.090**	0.094**	-0.123**	-0.177**
2		1	0.188**	-0.007	0.041	0.240**	0.522**	0.131**	0.083**	0.073**
3			1	0.654**	-0.093**	0.156**	0.077**	0.496**	-0.143**	0.335**
4				1	-0.136**	0.137**	-0.041	0.143**	-0.340**	0.143**
5					1	0.308**	0.074**	-0.042	0.495**	0.472**
6						1	0.472**	-0.003	0.394**	0.363**
7							1	0.091**	0.424**	0.420**
8								1	0.092**	0.163**
9									1	0.389**
10										1

*, ** Significant at 5% and 1%, respectively.

Jo 90 showed the earliest flowering landrace and shorter plant height at flowering. Also Jo 84, Jo 152 and Jo 155 flowered early and had short plant height at flowering.

4-1-3 Final Plant Height.

Final plant height among individual plants of nineteen okra landraces and the control cultivar "Clemson Spineless" ranged from 16-157 cm with an average of 58 cm and C.V. (31.3%) (Table 5 P. 26). Plants of landraces exhibited wide range for final plant height indicating the existence of variability among and within landraces. Selecting plants with medium final plant height and shorter internodes could be advantageous for higher yield. Final plant height with shorter internodes plays an important role in increasing the yield, as fruiting takes place at each node (Singh and Singh, 1979).

Significant differences in final plant height were showed among landraces (Table 6). Average final plant height ranged from 40 cm in Jo 79 to 73 cm in Jo 148 and Jo 157. Average final plant height in the control cultivar was relatively equal or shorter than landraces except for Jo 79 which registered lowest final plant height (40 cm). The very wide range between plants of each landrace including the control cultivar, and high value of C.V. are indicative of the effect of genetic and environmental factors on the final plant height. Plants of most landraces distributed over a

wide range showing heterogeneity in final plant height between plants of each landrace. Again, this heterogeneity may be due to the improper seed production that practiced by farmers.

Desirable heterosis over the mid-parent in final plant height was obtained from crosses between diverse parents (Singh and Singh, 1979). On the other hand, Jo 151 registered high value of C.V. (41.2%) and the lowest one (22.1%) was for Jo 152. Plant height is genetically controlled, which did not change irrespective of the environment (Raji, 1994).

Final plant height had positive and significant correlation with days to flowering (r = 0.090) and plant height at flowering (r= 0.522) (Table 7 P.31), indicating there is an association among these traits. Raji (1994) reported that cultivars with medium to tall height showed high yield. Plants of the Balady variety behave vigorous and taller than the rest varieties, and gave larger number of pods (Dewdar *et al.*, 1987). Landraces (for example, Jo 84, Jo 90 and Jo 152) which showed early flowering and short plant height at flowering produced medium final plant height.

4-1-4 Immature Pod Width.

Immature pod width among individual plants of nineteen okra landraces and the cultivar "Clemson Spineless" ranged from 0.67-1.9 cm with an average 0.97 cm and C.V. 10.5% (Table 5 P. 26). Significant differences in immature pod width were showed among landraces (Table 8) and the average immature pod width ranged from 0.85 cm in Jo 148 to 1.12 cm in Jo 151.

Means of immature pod width for the two landraces Jo 89 and Jo 151 were relatively higher than control cultivar but not significant. Individual plants in some landraces showed larger immature pod width than the control. This result indicates that single plant selection for larger immature pod width within landraces could be possible. Plants of Jo 88 and Jo 148 distributed over a wide ranged 0.73-1.90 cm and 0.67-1.60 cm, respectively (Table 8) indicating heterogeneity in immature pod width between plants for each landrace probably due to different genetic background of their parents. Generally, parents of different genetic background exhibited high heterotic value (Singh and Singh, 1979).

Some okra landraces behaved similarly in immature pod width such as Jo 149, Jo 157, Jo 90, Jo 150, Jo 147 and Jo 156.

The means of F_1 generation exceed the mean of the two parents (Jo 88, Jo 12) indicating that parents may posses different gene/s for immature

fruit diameter and continuos variation indicated that the immature pod width is quantitatively inherited (Salameh, 1998).

The relatively wide range for immature pod width 0.88-1.55 cm among plants of the control also indicated that environmental factors affect this trait. Immature pod width showed positive and significant correlation with days to flowering (r= 0.97) (Table 7 P. 31). Immature pod width showed negative correlation with plant height at flowering (-0.007) indicating weak association between these traits.

Table (8): Range (R), mean ($\bar{X}$), standard deviation (SD) and coefficient of variation (C.V.%) for immature pod width, immature pod length and mature pod length for nineteen okra landraces collected from different regions in Jordan and control grown in the Experimental Station at the University of Jordan Campus in 1998.

Landraces Number	Immature Pod width (cm)			Immature pod length (cm)			Mature pod length (cm)		
	Range	$\bar{X} \pm SD$	C.V.%	Range	$\bar{X} \pm SD$	C.V.%	Range	$\bar{X} \pm SD$	C.V. %
Jo 76	0.71-1.60	0.91 ± 0.09	9.9	2.9 - 5.9	3.9 ± 0.67	16.9	5 - 20	13 ± 6.5	48.6
Jo 79	0.90-1.25	1.05 ± 0.08	7.6	2.5 - 2.9	3.8 ± 0.68	12.8	6 -18	9 ± 4.1	47.2
Jo 80	0.67-1.14	1.02 ± 0.11	10.8	3.2 - 5.2	4.1 ± 0.66	16.1	3 -19	12 ± 6.2	54.6
Jo 84	0.72-1.60	0.87 ± 0.07	8.0	2.9 - 5.9	4.2 ± 0.67	16.1	7 -20	13 ± 6.4	49.9
Jo 88	0.73-1.90	0.95 ± 0.09	9.5	3.3 -5.9	4.6 ± 0.64	14.1	8 -19	13 ± 2.4	18.4
Jo 89	0.76-1.42	1.09 ± 0.15	13.8	2.7 - 5.4	3.5 ± 0.64	16.2	5 -19	10 ± 2.5	25.5
Jo 90	0.73-1.70	0.94 ± 0.10	10.6	2.9 - 5.8	4.1 ± 0.70	17.2	6 -17	12 ± 2.8	24.4
Jo144	0.76-1.20	0.97 ± 0.13	13.4	2.6 - 5.9	4.1 ± 0.77	18.7	6 -19	12 ± 2.4	21.1
Jo 147	0.75-1.90	0.92 ± 0.07	7.6	2.9 - 5.5	4.4 ± 0.68	15.5	9 -17	13 ±1.7	13.3
Jo 148	0.67-1.60	0.85 ± 0.08	9.4	2.9 - 5.5	4.1 ± 0.73	18.0	7 -20	13 ± 2.4	17.5
Jo 149	0.74-1.08	0.90 ± 0.08	8.9	2.9 - 5.4	4.1 ± 0.64	15.5	6 -19	13 ± 2.5	19.5
Jo 150	0.71-1.08	0.91 ± 0.08	8.8	3.3 - 5.9	4.3 ± 0.61	14.3	7 -17	13 ±2.5	18.8
Jo 151	0.86-1.79	1.12 ± 0.20	17.9	2.9 - 5.5	3.9 ± 0.67	17.1	6 -15	10 ±2.5	25.3
Jo 152	0.88-1.31	1.06 ± 0.09	8.5	2.9 - 5.4	4.0 ± 0.58	14.4	6 -16	11 ±2.4	21.4
Jo 154	0.80-1. 20	0.99 ± 0.09	9.1	2.9 - 5.9	4.1 ± 0.75	18.2	7 -20	12 ±2.0	16.5
Jo 155	0.83-1.27	1.03 ± 0.09	8.7	2.9 - 5.1	3.9 ± 0.64	16.3	5-18	12 ±2.3	20.3
Jo 156	0.75-1.11	0.92 ± 0.09	9.8	2.9 - 5.6	4.1 ± 0.61	15.1	8-16	13 ± 2	16.1
Jo 157	0.69-1.06	0.90 ± 0.08	8.9	2.9 - 5.9	4.4 ± 0.68	15.6	7-19	13 ±1.5	11.8
Jo 158	0.74-1.11	0.89 ± 0.08	9.0	2.9 - 5.7	4.0 ± 0.63	15.6	7-18	13 ±2.2	17.5
*Control	0.88-1.55	1.08 ± 0.11	10.2	2.9 - 5.5	3.9 ± 0.68	17.1	5-17	12 ±2.4	18.4
LSD at $p \leq 0.05$		0.058			0.338			1.62	

* = Clemson Spineless

Number of plants for each landrace = 60

4-1-5 Immature and Mature Pod Length.

Immature pod length among individual plants of nineteen okra landraces and the control cultivar "Clemson Spineless" ranged from 2.5-5.9 cm with an average 4.1 cm and C.V. 16.3% (Table 5 P. 26). Significant differences in immature pod length were observed among landraces (Table 8 P. 36) and average immature pod length was ranged 3.5 cm in Jo 89 to 4.46 cm in Jo 88. Average immature pod length was greater than control for example, Jo 80 and Jo 84 but not significant. The wide range in immature pod length and relatively higher C.V. may indicate the presence of variability. Immature pod length showed positive and significant association with days to flowering (r= 0.094), plant height at flowering (r= 0.131) and immature pod width (r= 0.143), indicating that selection one of these traits could be accurate estimate of the others, or improvement of one trait will cause simultaneous changes in the other traits.

Mature pod length among individuals of nineteen okra landraces and the cultivar "Clemson Spineless" ranged from 3.0-20 cm with an average of 11.9 cm and C.V. 20.9% (Table 5 P. 26).

Length of mature pod showed significant differences among landraces (Table 8) and average mature pod length ranged from 9 cm in Jo 79 to 13 cm in most landraces. Mature pods for plants of all okra landraces including control cultivar distributed over a wide range. These results indicate that trait is affected by environment. The highest C.V. (54.6%) was

for Jo 80 and the lowest one (11.8%) was for Jo 157. On the other hand Jo 148 and Jo158 showed the same values of C.V. (17.5%). In general, heritability estimates ranged from 20.3% to 78%, the low values may be due to low diversity between the parents or due to environmental effect (Salameh, 1998).

Mature pod length correlated positively and significantly with plant height at flowering (r = 0.083), and final plant height (r=0.424) indicating an association between the two traits exist. Mature pod length correlated negatively and significantly with days to flowering (r= -0.123) and pod width (r= -0.340) (Table 7 P. 31).

4-1-6 Immature Pod Weight.

Immature pod weight among individual plants of nineteen okra landraces and the cultivar "Clemson Spineless" ranged from 1.5-10 g with an average of 3.8 g and C.V. of 23.5% (Table 5 P. 26). The wide range for immature pod weight and relatively intermediate values of C.V. may indicate the existence of variability among and within landraces which allow for possible selection for proper immature pod weight.

Significant differences in immature pod weight were showed among landraces (Table 9). Average immature pod weight ranged from 3.04 g in Jo 148 to 4.41 g in Jo 151. Dewdar *et al.*, (1987) reported that Balady variety in Egypt produced small pods with lowest weight which preferred by consumer.

Table (9): Range (R), mean ($\bar{X}$), standard deviation (SD) and coefficient of variation (C.V.%), immature pod weight, number of pods/plant and pod yield. Plant for nineteen okra landraces collected from different regions in Jordan and control grown in the Experimental Station at the University of Jordan Campus in 1998.

Landraces number	Immature Pod weight (g)			Number of pods / plant			Pod yield/ plant (g)		
	Range	$\bar{X} \pm SD$	C.V.%	Range	$\bar{X} \pm SD$	C.V.%	Range	$\bar{X} \pm SD$	C.V.%
Jo 76	2.3-4.9	3.60 ± 0.78	21.5	5-39	19 ± 8.4	43.4	16-152	68 ±35.5	49.7
Jo 79	2.2-5.5	4.04 ± 0.80	19.8	3-26	11 ± 5.0	45.2	8-114	44 ±18.7	43.1
Jo 80	2.2-6.6	4.34 ± 0.95	21.9	2-34	13 ± 6.6	50.7	15-155	56 ±28.2	50.9
Jo 84	1.8-5.5	3.27 ± 0.64	19.6	3-32	17 ± 7.3	43.1	11-107	56 ±27.0	48.2
Jo 88	2.3-9.8	4.23 ± 1.05	24.8	3-24	13 ± 5.3	41.6	7-118	55 ±24.7	46.1
Jo 89	1.7-6.7	4.15 ± 1.05	25.3	4-45	13 ± 5.8	45.1	17-230	54±31.8	60.5
Jo 90	2.2-5.5	3.50 ± 0.83	23.7	4-46	16 ± 8.4	53.9	10 -159	56±28.8	53.4
Jo144	2.6-10	3.82 ± 0.89	23.3	5-31	13 ± 5.3	42.0	12-142	50±18.0	38.3
Jo 147	2.4-5.6	3.85 ± 0.81	21.0	3-38	15 ± 6.0	39.0	10-130	58±29.4	49.2
Jo 148	1.5-5.4	3.04 ± 0.68	22.4	2-36	17 ± 8.4	50.5	8-120	52±23.8	46.6
Jo 149	2.2-5.9	3.62 ± 1.04	28.7	4-47	16 ± 8.7	54.4	14-139	58±32.5	56.7
Jo 150	2.2-5.6	3.61 ± 0.85	23.5	3-46	15 ± 6.8	45.3	10-75	54±23.4	44.1
Jo 151	2.1-10	4.41 ± 1.46	33.1	5-37	15 ± 7.6	50.6	12-155	66±31.1	48.9
Jo 152	2.8-6.4	4.37 ± 0.78	17.9	4-37	11 ± 5.9	53.0	14-167	48±27.7	55.5
Jo 154	2.2-7.0	3.99 ± 1.00	25.1	4-58	16 ± 6.4	41.0	20-211	64±33.4	54.6
Jo 155	2.4-6.4	4.21 ± 0.90	21.4	4-40	14 ± 6.1	43.0	17-130	59±28.2	48.1
Jo 156	2.4-5.6	3.51 ± 0.69	21.9	4-30	13 ± 6.5	48.6	12-104	46±42.9	53.1
Jo 157	2.0-5.6	3.71 ± 0.70	18.9	3-48	16 ± 7.9	48.8	13-186	59±33.2	55.0
Jo 158	2.3-5.0	3.39 ± 0.61	18.0	4-48	15 ± 7.7	52.3	9-158	51±28.9	57.9
*Control	2.9-7.5	4.14 ± 0.78	18.8	4-46	16 ± 8.3	52.7	15-199	66±41.4	61.8
LSD at $p \leq 0.05$		0.479			4.46			19.56	

* = Clemson Spineless

Number of plants per landrace = 60

The relatively wide range for immature pod weight (2.9-7.5) cm among plants of the control also indicated that environmental factors affect this trait. The low heritability estimates for immature pod weight demonstrated the instability of response to environment (Ariyo, 1990).

Immature pod weight showed positive and significant association with days to flowering (r= 0.056), pod width (r= 0.654), final plant height (r= 0.522) and immature pod length (r= 0.496) and plant height at flowering (r = 0.188) and negative association with mature pod length (r= -0.143) and number of pods/plant (r = -0.093) (Table 7 P. 31). Immature pod weight had positive association with pod width and it is considered to be the most reliable index for pod yield (Ariyo, 1990).

4-1-7 Number of Pods per Plant

Number of pods per plant for individual plants of nineteen okra landraces and the cultivar "Clemson Spineless" ranged from 2-58 with an average of 15 and C.V. of 50.2% (Table 5 P. 26). Number of pods per plant showed significant differences among landraces and the average pods per plant ranged from 11 in Jo 79 to 19 in Jo 76 (Table 9 P. 39). For each landrace, number of pods per plant showed very wide range and the C.V. was very high and similar to the control cultivar which showed also wide range (4-46) and very high C.V. (52.7%). This result demonstrates that variability within individuals may be due to environmental effects.

However, variation among landraces is an indication of genetic influence in this trait.

Plants of Jo 154, Jo 157 and Jo 158 distributed in a wider range of 4-58, 3-48 and 4-48, respectively. This indicates heterogeneity in number of pods per plant and among plants for each landrace. Number of pods per plant showed low heritability estimate which supports the idea that environmental factors affect this trait (Ariyo, 1990). Number of pods per plant correlated positively and significantly with final plants height (r=0.074) (Table 7 P. 31), The longer the plants are the more number of pods per plant are obtained. Also selection for one trait could be an accurate estimate of the others. On the other hand, from the number of pods per plant number of nodes would be know which indicate to the total pod yield per plant.

Negative correlation was observed between number of pods per plant and days to flowering (r = -0.194), pod weight (r= -0.093), pod width (r=-0.136) and immature pod length (r= -0.042). The negative association between earliness and number of pods per plant may indicate that delay in flowering and consequently delay in harvesting may develop vigorous plants which produce higher number of pods per plant. Similar explanations may be applied to the negative association of number of pods per plant with width, length and weight of pods. Ariyo (1987) reported also

negative correlation of number of pods per plant with days to flowering (r= -0.23) and immature pod length (r= -0.12).

4-1-8 Yield of Pods Per Plant.

Pod yield per plant for individual plants of nineteen okra landraces and the control cultivar "Clemson Spineless" ranged from 7-230 g with an average of 56g and C.V. of 35.7% (Table 5 P. 26). Significant differences in pods yield per plant were found among landraces and the average pods per plant ranged from 44g in Jo 79 to 68g in Jo 76 (Table 9 P. 39).

The Jo 76 gave the highest average pods yield per plant (68 g) followed by control cultivar (66 g). The lowest value was 44 g for Jo 79. The very wide ranges and too high C.V. and S.D. for yield per plant for all tested landraces including the control cultivar indicate large variations. It is difficult to judge whether these variations are due to environment, genetic and / or environment and genotype interaction. However, in general, yield is a complex trait and it is affected by genetic and environment but the magnitude of this influence can not be estimated by these statistical parameters. Such a complex traits, like yield, require repeating the evaluation under different locations and for more than one season. Kaul *et al.*, (1978) reported that selection for plant height and pods per plant would result in the selection of high yielding genotypes. Cross-combination involving diverse parents gave higher yields (Singh *et al.*, 1977).

The fact that edible pod yield per plant and days to flowering accounted 57.83% of total genetic diversity indicating that the two characters would be reliable to distinguish among okra varieties (Ariyo, 1987).

Pods yield per plant correlated positively and significantly with plants height at flowering (r= 0.073), immature pod width (r= 0.143), immature pod length (r= 0.163), mature pod length (r= 0.389), pod per plant (r= 0.472) and pod weight (r=0.335) (Table 7 P. 31). These traits which showed association with yield are considered components of yield and any positive improvement of these traits will contribute to the increase of yield. Ariyo (1987) reported that immature pod weight, immature pod length, immature pod width, pods per plant and length of mature pods showed significant genotypic correlation with pod yield per plant. In addition, selection for any of these traits would be resulted in the selection of high yielding genotypes. On the other hand, number of days to flowering had a negative correlation with yield of pods per plant (r= -0.177) indicating that earlier crops produce a higher yield. Ariyo (1987) and Raji (1994) reported that number of days to flowering had a large direct effect on pod yield with its largest indirect effect through reduction in edible pod weight.

4-1-9 Seed Per Pod

Seed per pod for individual plants of nineteen okra landraces and the cultivar "Clemson Spineless" ranged from 8-115 with an average of 61 and C.V. of 25.5% (Table 5 P. 26). Significant differences in seed per pod were found among landraces (Table 10) and the average seed per pod ranged from 50 in Jo 84 to 68 in Jo 151.

The highest C.V. (32.8%) was for Jo 154 and the lowest one (17.2%) was for Jo 150. The wide range of seed per pod (14-103) and relatively high C.V. in tested landraces including the control cultivar suggests the influence of the environment on this trait.

Seed per pod correlated positively and significantly with, plant height at flowering (r = 0.240), final plant height (r=0.472), pod width (r=0.137), mature pod length (r=0.394) and pod yield per plant (r= 0.363) (Table 7 P. 34). Since seed yield and pod yield were highly correlated, any program for the improvement of pod yield would result in the improvement of seed yield also (Kaul and Peter, 1978).

Strong association showed in this study indicating selection one trait could be accurate estimate of the others. Low correlation observed between seed per pod and immature pod length, indicating weak association between the two traits.

Table (10): Range (R), mean ($\bar{X}$), standard deviation (SD) and coefficient of variation (C.V.%) of seed per pod for nineteen okra landraces collected from different regions in Jordan and control grown in the Experimental Station at the University of Jordan Campus in 1998.

Landraces number	Seed / pod		
	Range	$\bar{X} \pm SD$	C.V.%
Jo 76	38-91	66 ± 15.4	23.4
Jo 79	37-90	63 ± 15.6	24.6
Jo 80	12-103	63 ± 18.2	28.6
Jo 84	21-97	50 ± 14.3	28.3
Jo 88	38-96	63 ±11.9	18.8
Jo 89	16-93	62 ±17.5	28.5
Jo 90	16-84	55 ±14.8	26.8
Jo144	12-93	59 ±14.5	24.5
Jo 147	22-89	63 ±12.0	18.9
Jo 148	26-97	57 ±14.8	26.2
Jo 149	16-83	58 ±13.8	23.9
Jo 150	34-94	63 ±10.9	17.2
Jo 151	26-115	68 ±17.4	25.4
Jo 152	26-95	60 ±16.5	27.6
Jo 154	25-104	57 ±18.8	32.8
Jo 155	22-98	61 ±19.1	31.1
Jo 156	29-97	56 ±13.1	23.3
Jo 157	19-99	61 ±11.8	19.3
Jo 158	8-87	59 ±13.3	22.4
*Control	14-103	66 ±17.3	26.4
LSD at $p \leq 0.05$		9.39	

Number of plant per landrace = 60

4-1-10 Stem, Leaf, Petiole, and Pod Color.

Stem, leaf, petiole and pod color of nineteen okra landraces and the cultivar "Clemson Spineless" are presented in Table 11.

Variation among and within okra landraces in stem color is expressed in scores, 1 = red, 2= green, 3= green with red patches, 4= purple. Plants of twelve landraces showed purple stem color, one landrace and the control cultivar had stem of green with red patches; plants of four landraces had either purple or green with red patches stems and one landrace has green stem color in addition to purple and green with red patches. However, one landrace (i.e Jo 148) was unique where all plants except two showed red stem color. These results indicate that this trait is under genetic control. The variation in stem color within some landraces is due to pollen contamination and improper isolation of plants used for seed collection. Plants of F_1 and F_2 generations resulted from crossing between two red stem parents (Jo 12 and Jo 49) showed red stem color indicating that genes controlling stem color in both parents are allelic to each other (Salameh, 1998). Crossing between green stem parent and red patches parent, produced plants with only red patches stems suggesting dominance of red patches stem over green stem (Salameh, 1998). Homogeneity in stem color of the control cultivar suggests purity of seeds.

The variation within and among okra landraces of leaf color and petiole expressed in scores as following: 1=red, 2=green, 3= green with red

Table (11): Number of plants showing different stem, leaf, petiole and pod color for nineteen okra landraces collected from different regions in Jordan and control grown in the Experimental Station at the University of Jordan Campus in 1998.

Landraces number	Stem color				Leaf color			Petiole color	Pod color			
	1 = Red	2 = Green	3 = Green with red patches	4 = Purple	1 = Red	2= Green	3= Green with red veins	1 = Red	1 = Red	2 = Green	3 = Green with red patches	5 = Yellowish green
Jo 76				60			60	60		4	46	10
Jo 79		3	37	20		60		60		59		1
Jo 80			40	20		60		60		58	1	1
Jo 84				60			60	60			57	3
Jo 88				60			60	60		16	20	14
Jo 89			20	40			60	60		19	6	35
Jo 90				60			60	60		7	45	8
Jo 144				60			60	60		7	45	8
Jo 147				60			60	60		25	23	12
Jo 148	58			2	58		2	60	58			2
Jo 149				60			60	60		8	49	3
Jo 150				60			60	60			60	
Jo 151				60			60	60		32	14	14
Jo 152			60			60		60		40	20	
Jo 154			20	40		60		60		36	22	2
Jo 155			40	20		60		60		52	3	5
Jo 156				60			60	60		5	52	3
Jo 157				60			60	60		12	39	9
Jo 158				60			60	60		8	42	10
* Control			60			60		60		60		

* = Clemson Spineless
Number of plants / landrace = 60

color of stem, leaf and pod, indicating the genetic purity of this landrace for the color. The other landraces showed variations among and within plants. Control cultivar produced only green pod indicating that green color is under genetic control without the effect of the environment.

Salameh (1998) reported that crossing between two parents one with red pod color and other green pod color gave F_1 progeny with red color, suggesting complete dominance of red over green color. Most plants of landraces showed pods of green with red patches. Kalia and Padda (1962) reported multiple allelic series appeared to control fruit color, green being dominant over cream, while purple was dominant over green and cream.

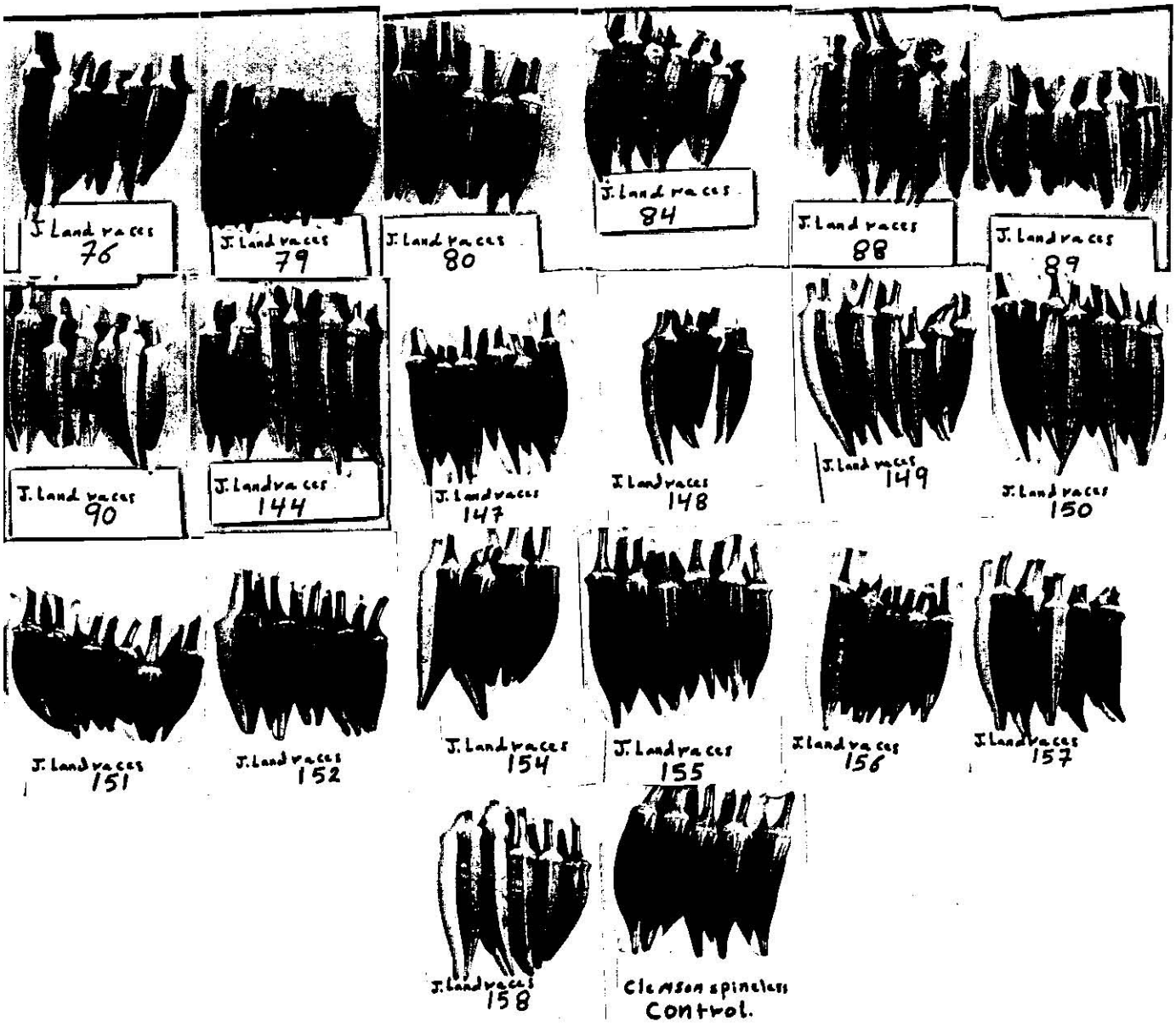

Plate1: Variation in Pod Characteristic of Okra Landraces Collected from Different Regions in Jordan in 1995.

5- RAPD Marker Characterization of Okra Landraces.

5-1 Quantity and Quality of Extracted DNA.

Genomic DNA quality and quantity of five okra landraces and "Clemson Spineless" cultivar was visually checked by agarose electrophoresis (Fig. 1). Quality and quantity of isolated okra DNA's were variable (Fig. 1). Variability in the amount of DNA was clear between plants from the same or different landraces; for example, the two plants of Jo 156 and one plant from Jo 148 show little amount of DNA. This indicates that the used amount of DNA (5 µl) is not enough to detect for certain okra landraces while others yielded enough amounts to proceed in RAPD. Moreover, DNA preparations could contain some impurities such as salts which interfered in DNA mobility and result in strikes for DNA bands.

DNA concentrations (ng/µl) were measured using flourometry (Table 3). Approximately similar concentrations of the different isolated DNA within and among okra landraces are shown. Comparing what obtained using ethidium bromide stained gel (Fig. 1) and flourometer measurement (Table 3), indicates that these two methods do not produce same results. For example, the same plant from Jo 148 visually shows relatively high amount of DNA but at the same time the DNA concentration registered for the same plant was equal to 31 ng/ µl which was the highest concentration.

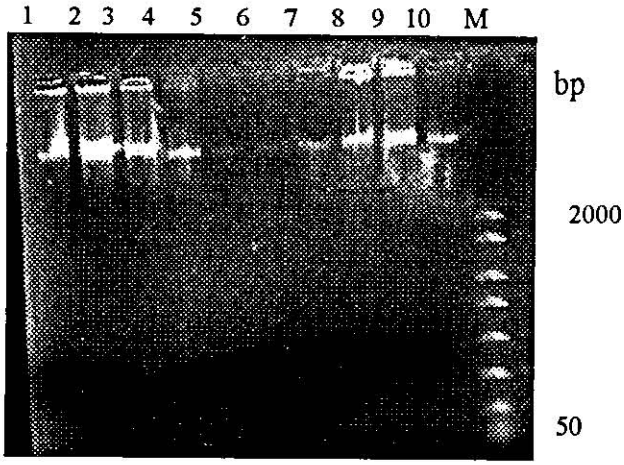

Fig.1:Detecting genomic DNA from different plants for some okra landraces and one control cultivar using 0.7% agarose gel stained with ethidium bromide. Lane 1-2 Clemson Spineless; lane 3-4 (Jo 84); lane 5-6 (Jo 156); lane 7-8 (Jo 148); lane 9-10 (Jo 154); lane M: PCR marker (Sigma).

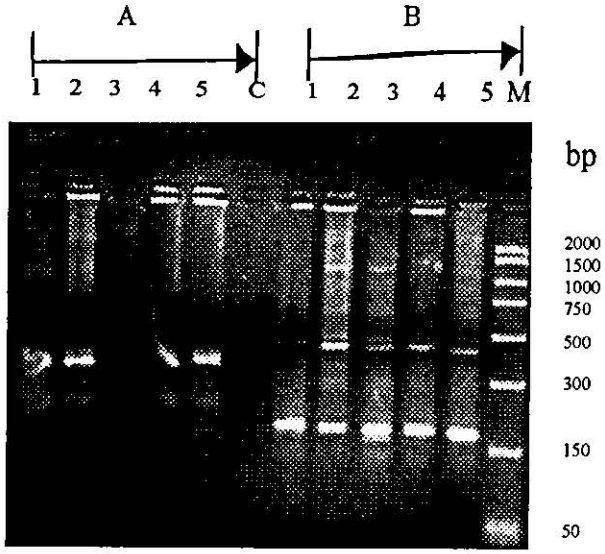

Fig 2:Preliminary RAPD analysis generated in a single PCR with different primers from different plants of okra landraces and one cultivar. A-RAPD products generated with the primers (OPA02, OPA10 and OPA19). Lane 1: Clemson Spineless; lane 2: (Jo 148); lane 4: (Jo 148); lane 5: (Jo 154); lane M: PCR marker(Sigma);lane C:negative control (without DNA template).B-RAPD products generated with the primers (OPA02, OPA10 and OPA13).Lane1: Clemson Spineless; lane 2-3: (Jo 84); lane 4: (Jo 148); lane 5: (Jo 154); lane M: PCR marker (Sigma); lane C: negative control (without DNA template).DNA samples were separated on 1.4 % agarose gel and stained with ethidium bromide.

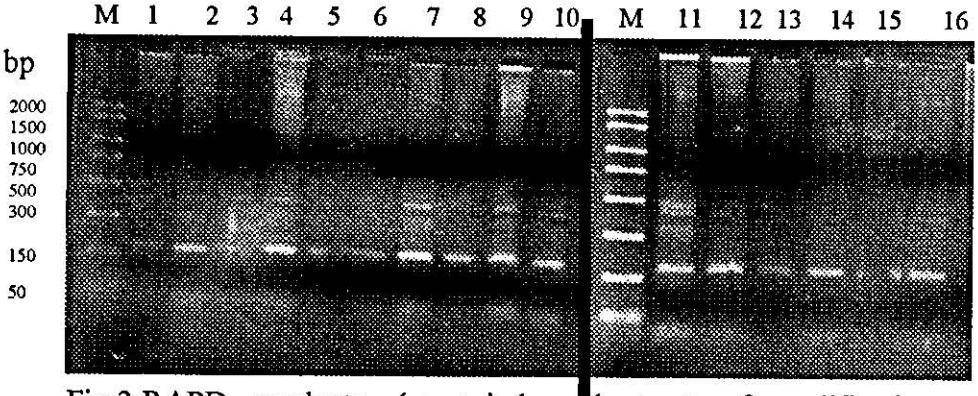

Fig.3:RAPD products (two independent sets of amplification and electrophoresis) generated by using primers (OPA02, OPA09 and OPA13) within four Jordanian okra landraces and separated on 1.4% agarose gel stained with ethidium bromide. Lane 1-4 (Jo 154); lane 5-8 (Jo 148); lane 9-12 (Jo 84); lane 13-16 (Clemson Spineless); lane M: PCR marker (Sigma).

Polysaccharides which are a long chain molecules of sugars increased in amounts with age of okra plants and interfere in DNA isolation (Dekochko and Hamon, 1990). Reducing the interference of polysaccharide in DNA extraction in this study was obtained by using true leaves from two week old seedlings and using diluted 2 X CTAB extraction buffer into 1.33 X CTAB (Appendix B 1-2). While Dekochko and Hamon (1990) used cotyledons of seedlings which was grown in dark to purify total DNA from the genus *Abelmoschus*.

5-2 Reproducibility and DNA Size Determination.

The reproducibility is an important factor for determining the usefulness of RAPD technique and detection of the genetic variation. Reproducibility was checked by repeating amplification on some individual DNA of okra landraces using different random primers. Only reproducible bands were scored as either present or absent. The only RAPD marker scored that are equal or less than 1000 bp in size, while RAPD marker more than 1000bp were neglected due to inconsistency observed in independent amplification (Fig. 2 and 3). Evaluating only consistently amplified fragments and excluding variation in minor bands were previously reported by Gregor *et al.*, (1994).

Sizes of each RAPD marker were determined by a standard curve using logarethmatic scale versus mobility of DNA (Fig. 6). The band

mobility from wells runs on agarose gel facilitate the measurement of band molecular weight (bp) using molecular weight marker.

5-3 Genetic Relationship Among and Within Okra Landraces.

Twenty RAPD primers were used to amplify the DNA of the five okra landraces and Clemson Spineless (Table 4). Twelve primers including, OPB05 and OPM05, failed to produce any clear fragments and some others produced smeared patterns. This could indicate that a high mismatch between primers and DNA template prevented production of specific bands. Ho *et al.* (1997) reported that the primers OPB05 gave no amplification products when used to investigate relationships among 18 *Cynodon* cultivars in Australia.

Eight primers including OPA02, OPA09 and OPA13 out of 20 random primers gave reproducible banding patterns (Fig. 2 and 3). Figure 2 illustrates limited number of amplified products using DNA from okra landraces and control. The RAPD marker profiles were similar within each okra landraces and control using primers OPA02, OPA10 and OPA19 (Fig. 2A), primers OPA02, OPA10 and OPA13 (Fig. 2B) and primers OPA02, OPA09 and OPA13 (Fig. 3).

Figure 4 shows RAPD patterns obtained using the single primer OPA13 for different plants among five okra landraces collected from different regions in Jordan and Clemson Spineless. RAPD marker profiles

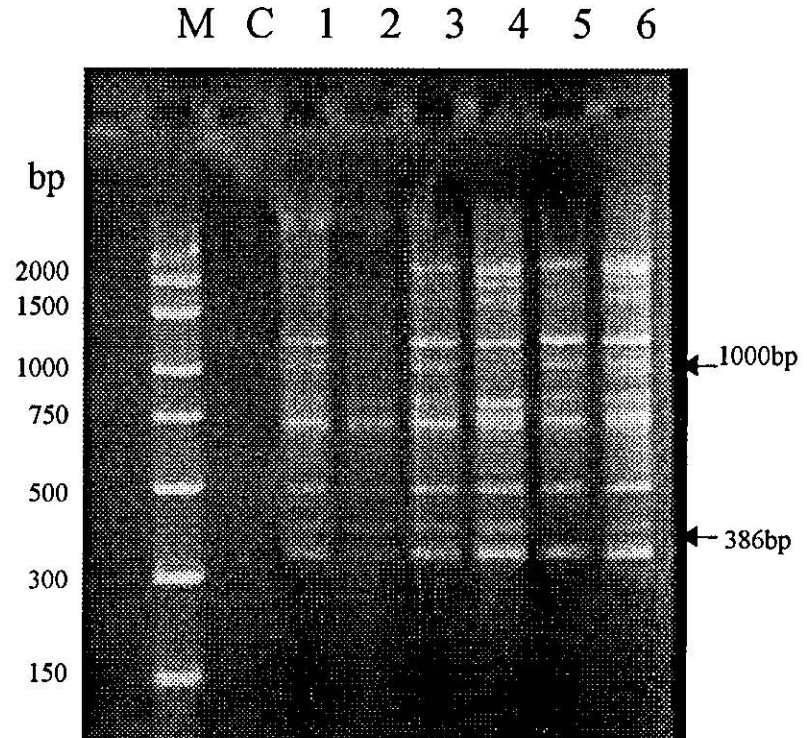

Fig.(4):RAPD patterns obtained among five okra landraces collected from different regions in Jordan and commercial cultivar "Clemson Spineless" using primer OPA13.Lane1:Clemson Spineless;lane2:Jo 84;lane3:Jo 148 ;lane 4:Jo 154;lane 5: Jo 76;lane 6:Jo 156. Lane M : PCR marker (Sigma) ;lane C: negative control (without DNA template).Marked bands are polymorphic among okra landraces.

contained around 10 bands using the single OPA13 primer. Using combination of three primers including OPA13 (Fig. 2) produced only 2-3 bands. This was interesting in that increasing the number of primers resulted in less bands produced. Figure 5 also shows RAPD pattern obtained by OPA13 within five okra landraces and Clemson Spineless and shows similar results to Fig. 4. All okra landraces and Clemson Spineless show similar banding pattern using OPA13 with few exceptions.

Minor differences in banding patterns were seen for some okra landraces; for example, landrace Jo 76 does not produce the band with molecular weight 386 bp, and Jo 154 does not produce the band with molecular weight 1000 bp with primer OPA13 (Fig. 4). These landraces could be considered genetically different but the absence of this band should not be considered as a characteristics of these landraces unless all other landraces were tested.

Figure 4 and Figure 5 show RAPD results of the primer OPA13 where the band (386 bp) was absent in okra landrace Jo 76, and present in Jo 156, Jo 154, Jo 148, Jo 84 and Clemson Spineless. This indicates that the primer OPA13 amplified different loci in okra.

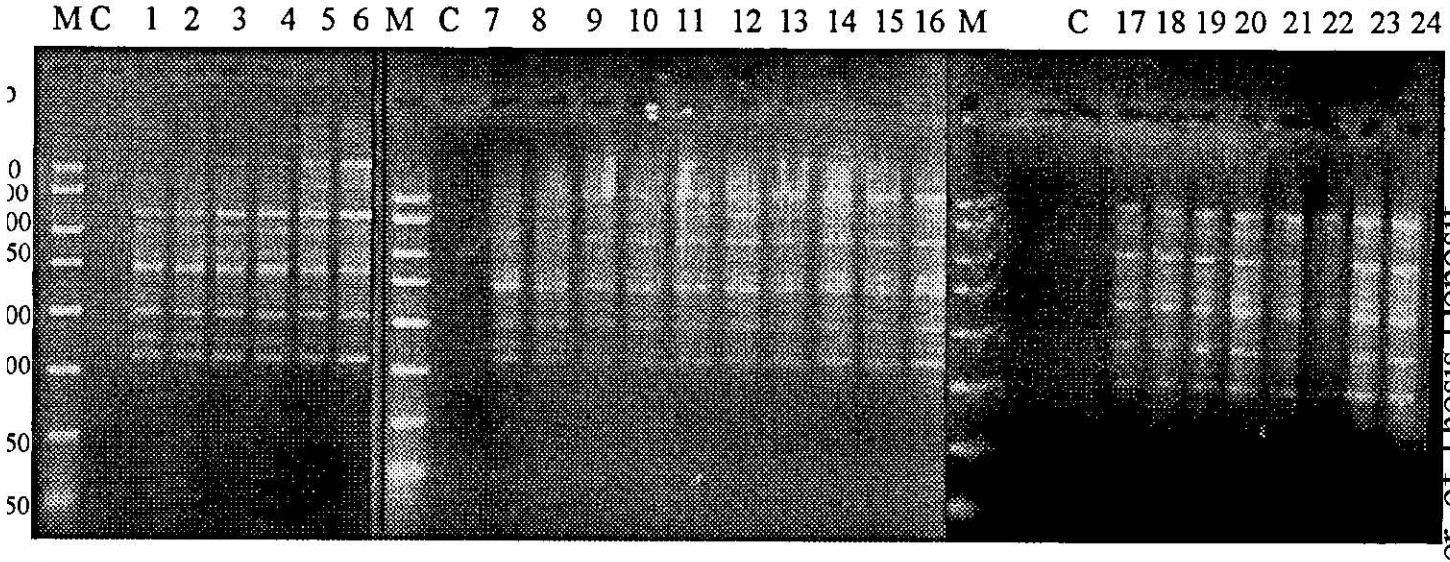

Fig .(5):RAPD patterns (three independent sets of PCR amplification and electrophoresis) obtained within Jordanian okra landraces and commercial cultivar "Clemson Spineless" using primer OPA13.Lane 1-4 (Clemson Spineless); lane 5-8 (Jo 84);lane 9-12 (Jo 148); lane 13-16 (Jo 154);lane 17-20 (Jo 76);lane 21-24 (Jo 156).LaneM:PCR marker (Sigma);C:negative control (without DNA template).

Table (12): Number of total bands produced (≤ 1000 bp) among Jordanian okra landraces and Clemson Spineless cultivar from independent amplified PCR.

Landraces	Among landraces	Among landraces
	Fig. 3 OPA (O2, 10, 13)	Fig.4 OPA13
Jo 76	-	5
Jo 84	6	6
Jo 148	2	6
Jo 154	3	5
Jo 156	-	6
* Control	3	6

(*) Control = Commercial cultivar "Clemson Spineless"

(-) Not used in the PCR amplification

Data in Figure 5 were converted to Table 13. The band with molecular weight 386 bp was absent in some okra landraces, and present in the others suggesting that this band could be considered a landrace specific RAPD marker to discriminate okra landraces, after confirming lack of this band in the remaining not tested landraces. Single, unique DNA marker was used to identify different species of Heliconia (Kumar, 1998).

Table (13): Representation of RAPD markers in five Jordanian okra landraces and Clemson Spineless cultivar using primer OPA13 from independent amplified PCR.

Amplified fragment (bp)	Fig. 4						Fig. 5					
	Jo 76	Jo 84	Jo 148	Jo 154	Jo 156	Control	Jo 76	Jo 84	Jo 148	Jo 154	Jo 156	Control
386	-	+	+	+	+	+	-	+	+	+	+	+
394	+	+	+	+	+	+	+	+	+	+	+	+
500	+	+	+	+	+	+	+	+	+	+	+	+
744	+	+	+	+	+	+	+	+	+	+	+	+
830	+	+	+	+	+	+	+	+	+	+	+	+
1000	+	+	+	-	+	+	+	+	+	-	+	+

*Control: Clemson Spineless

(+) Present band

(-) Absent band

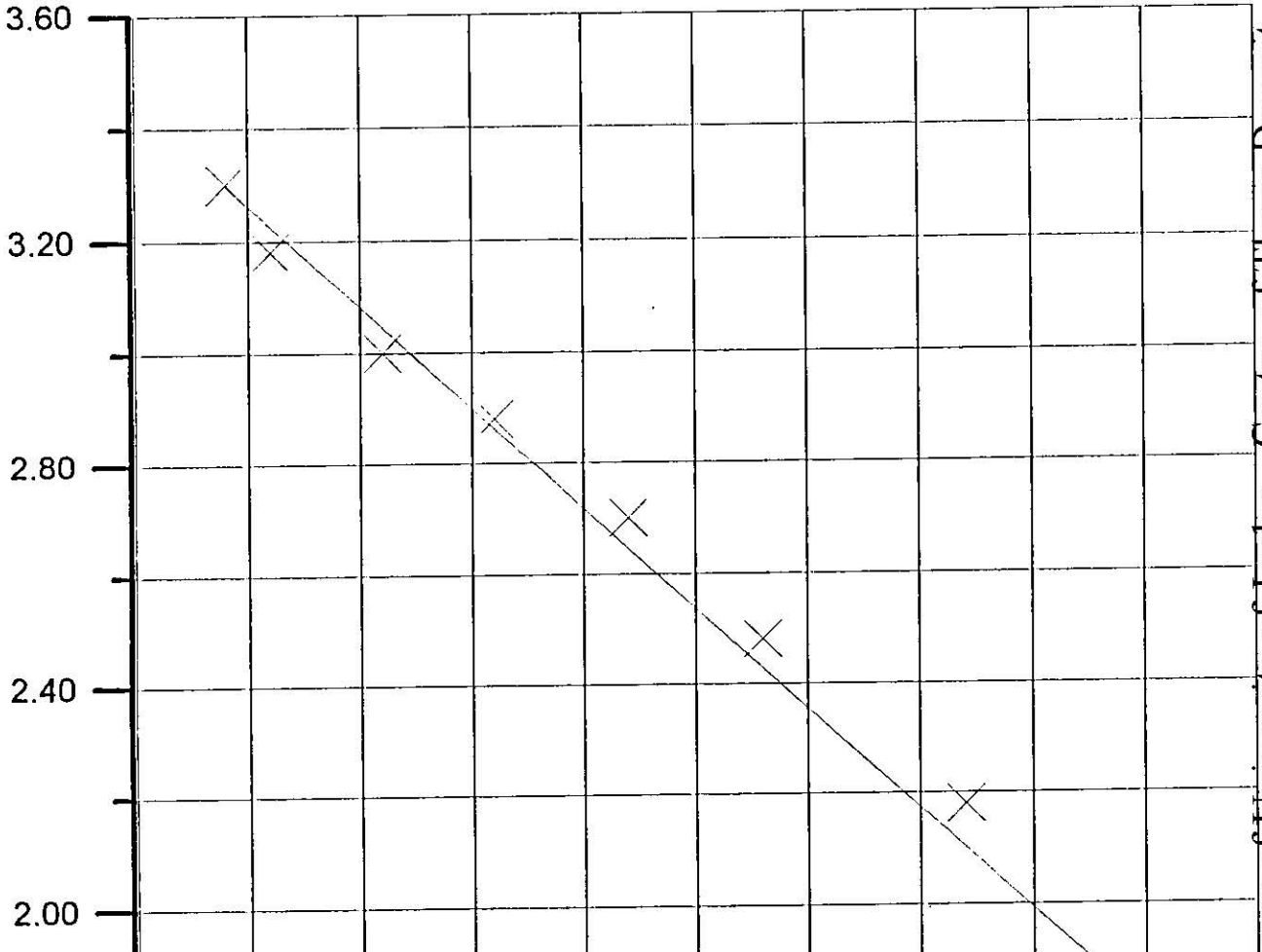

Fig. (6) :Example of a standard curve used to determine DNA mobilities fro the origin and sizes of DNA bands in agarose gel electrophoresis.

The amplification products obtained with the primer OPA13 were analyzed pairwise to compare genetic relationships among okra landraces (Table 14). Based on similarity matrix, it was difficult to find significant differences within okra landraces. Among the individuals of okra landraces collected from different regions in Jordan, highest similarity values estimated bands with molecular weight less than 1000 bp was registered as (1.000), while the lowest similarity was (0.909).

High similarity could be due to that the landraces are derived from the same population with a common ancestor. Also the control cultivar registered high similarity index (1.000) with some landraces. This could be due to that their seeds were derived from the same parents. This type of analysis could give clues about how and where okra landraces were distributed among growers.

Table (14): Similarity matrix among five okra landraces collected from different regions in Jordan and "Clemson Spineless".

Landraces	Jo 76	Jo 156	Jo 154	Jo 148	Jo 84	Clemson Spineless
Jo 76	1.000	0.909	1.000	0.909	0.909	0.909
Jo 156		1.000	0.909	1.000	1.000	1.000
Jo 154			1.000	0.909	0.909	0.909
Jo 148				1.000	1.000	1.000
Jo 84					1.000	1.000.
* Control						1.000

* Control: Clemson Spineless

5-4 Comparing Morphological Traits with RAPD Markers.

5-4-1 Variations among okra landraces and control cultivar.

Okra landraces that were collected from different regions in Jordan showed significant differences in morphological traits (see Section 4). Jo 76 okra landrace was collected from Irbid Fou'rra-Hawar junction and Jo 154 was collected from Madaba-Gernatah. Both landraces show similar quantitative and qualitative traits; for example days to flowering and green pod color, respectively. On the other hand, Jo 76 and Jo 154 show significant differences in quantitative and qualitative traits compared to other okra landraces (Jo 156, Jo 148, Jo 84) and control cultivar; for example, Jo 148 have red stem and pod color while others have green with red patches. At the DNA level, Jo 76 and Jo 154 show RAPD marker

profile containing one more band compared to the number in the RAPD marker profile of others. These additional bands were obtained by a single primer (OPA13) and appeared unlinked to the tested morphological trait. Other genetic makeup differences could have produced this polymorphism which could be used to discriminate between these two landraces.

Similarity index 1.000 was found in pairwise analysis of each two okra landraces. For example, Jo 84 with control cultivar and Jo 156 with Jo 84 (Table 14) show high value of similarity index. Comparing control cultivar with okra landraces for example, Jo 84 and Jo 156, shows high value of similarity (1.000), indicating these landraces could have similar genetic makeup but further assays with other primer should be done.

5-4-2 Polymorphism Within Okra Landraces and Control Cultivar.

Variations within each landrace in morphology were limited indicating homogeneity of tested landraces and the cultivar. This could be explained by the self pollinated nature of okra. In addition, landrace seed could have been collected from few parents or the selected regions were exposed to the same ecogeographical conditions. Also, the hybrid cultivar showed no internal morphological variation as it was expected.

RAPD marker profile also confirmed the absence of variation within plants of control cultivar (Fig. 5), indicating that the hybrid cultivar have homogenious of seed that give plants with similar genetic makeup. On

the other hand, other primers may be assayed to detect if DNA polymorphism present within plants of this cultivars in the future. The small morphological variations that appeared among okra landraces and control could be based on the differences in the genetic makeup of either multigenes or single genes.

In addition, no variation at the DNA level was detected by RAPD technique within Jordanian okra landraces and control cultivar using the three primers together OPA02, OPA09 and OPA13 or the single primer OPA13 (Fig. 3). Consistency in RAPD marker profiles for bands equal or less than 1000 bp was confirmed for the four plants in each landrace (Fig. 5). At the DNA level, high similarity within plants of each landrace, indicates that these plants could have similar genetic makeup. On the other hand, absence of variation within landraces could mean that other primers could be assayed in the future to check for polymophism within landraces.

6- Conclusions

1- Landraces are considered as a source of desirable traits, which can be used in breeding programs.

2- Morphological traits are used as the base for knowing and distinguishing among and within collected genetic resources or cultivars.

3- Days to flowering, number of pods per plant, immature pod width, pod color and pod shape showed high variability and could be used to discriminate between genotypes.

4- Pod yield per plant is positively associated with number of pods per plants, pod width, immature pod weight and final plant height, So improvement of these characters would result in the improvement of the others.

5- DAN fingerprinting based on PCR could be a suitable technique to facilitate detection of genetic variation.

6- RAPD technique could be useful for estimating genetic variability among landraces and cultivar.

7- DNA-fingerprinting-PCR can be assayed at the seedling stage, permitting one to make selection before many traits can be seen, thus reducing the number of individuals, which must be grown to maturity.

8- Okra landraces showed high morphological variations, but the variation in DNA level, was not enough to clearly distinguish between them.

9- With a high caution only Jo 76 could be distinguished from other okra landraces using DNA fragment size of 386 bp and primer OPA13.

7- References:

- Ariyo, O.J., and Aken'Ova, M.E. 1986. Evaluation of Varieties of Okra (***Abelmoschus esculentus*** **(L.)** for Distinctness and Uniformity. *Nigerian Journal of Agronomy*, **1** (3): 97-102.
- Ariyo, O.J., Aken' Ova, M. E. and Fatokun, C.A. 1987. Plant Character Correlation's and Path Analysis of Pod Yield in Okra (***Abelmoschus esculentus L.)***. *Euphytica*, **36**(3): 677-686.
- Ariyo, O.J. 1990. Effectiveness and Relative Discriminatory Abilities of Techniques Measuring Genotype X Environment Interaction and Stability in Okra (***Abelmoschus esculentus L. Monech)***. *Euphtica,* **47**: 99-105.
- Ariyo, J.O. 1990. Measurement and Classification of Genetic Diversity. in Okra (***Abelmoschus esculentus L.***). *Ann. Appl. Biol*, **116**: 335-341.
- Ariyo, O.J. 1987. Multivariate Analysis and the Choice of Parents for Hybridization in Okra *(**Abelmoschus esculentus L. Monech)**. Theor. Appl. Genetic,* **74**: 361 – 363.
- Ariyo, O.J. 1990. Variation and Heritability of Fifteen Character in Okra (***Abelmoschus esculentus L. Monch***). *Trop. Agri (Trindad)*, **67**(3): 213-216.
- Bisht, I.S., Patel, D.P., and Mahajan, R.K. 1997. Classifications of Genetic Diversity in (***Abelmoschus tuberculatus L.)***

Germplasm Collection using Morphometric Data. *Ann. Appl. Biol*, **130**: 325-335.

- Charrier, A. 1984. Genetic Resources of the Genus ***Abelmoschus*** med. (Okra). International Board for Plant Genetic Resources, Rome, 52- 56.

- Chheda, H. R and Fatokun, C.A. 1990. Studies on Okra Germplasm in Nigeria. Report of an international Workshop on Okra Genetic Resources Held at the National Bureau for Plant Genetic Resources, New Delhi, India, **8-12** October , 21-23.

- Degani, C., Rowland, L.J., Levi, A., Hortynski, J.A. and Galletta, G. J. 1998. DNA Fingerprinting of Strawberry (***Fragria X ananassa***) Cultivars using Randomly Amplified Polymorphic DNA (RAPD) Markers. *Euphytica,* **102:** 247-253.

- Department of General statistics, Statistical year book. Amman 1995.

- Department of General statistics, Statistical year book. Amman 1996.

- Department of General statistics, Statistical year book. Amman 1997.

- Dewdar, S.A., Atia, H., M. and El-Abd, M.T. 1987. Evaluation of some Okra Varieties under Cover and Open Field Conditions. *Al-Azhar. J. Agric. Res*, **8:** 53-64.

- Dollo, L., Hormaza, J.I. and Polito,V.S.1995. RAPD Polymorphisms Among Pistachio ***(Pistacia vera L.)*** Cultivars. *Fruit Varieties Journal*, **49**(3): 147-152.

- Dekochko, A. and Hamon, S. 1990. A rapid and Efficient Method for the Isolation of Restrictable Total DNA from Plants of the Genus *Abelmoschus*. *Plant. Mole. Biol. Rep,* **8**(1): 3-7.

- Doyle, J.J. and Doyle, J.L. 1990. Isolation of Plant DNA from Fresh Tissue. *Focus*, 12: 13-15.

- El-Tahir, I.M. 1990. Okra Genetic Resources in Sudan. Report of an international Workshop on Okra Genetic Resources Held at the National Bureau for Plant Genetic Resources, New Delhi, India, **8-12** October, 34-35.

- Erickson, H.T. 1962. Inheritance of Four Plant and Floral Characters in Okra (***Hibiscus esculentus L.***). *Amer. Soc. Hort. Sci*, **83**: 605-609.

- Farnham, M.W.1996. Genetic Variation Among and Within United States Collard Cultivars and Landraces as Determined by Randomly Amplified Polymorphic DNA Marker. *J. Amer. Soc.Hort. Sci,* **121**(3): 374-379.

- Fatokun, C.A. 1987.Wide Hybridization in Okra. *Theor. Appl. Genet*, **74:** 483-486.

- Ferguson, M.E., Robertson, L.D., Ford-Loyed, B.V., Newbury, H.N. and Maxted, N.1998. Contrasting Genetic Variation Amongst Lentil Landraces from Different Geographical Origins. *Euphytica*, **102:** 265-273.

- Foolad, M.R., Jones, R.A. and Rodriguez, R.L. 1993. RAPD Markers for Constructing Intraspecific Tomato Genetic Maps. *Plant Cell Reports*, **12**(5): 293-297.

- Frankel, O.H. 1977. *Natural Variation and its Conservation in Muhammed,* .R. Askel and R.C.Von Borstel (Eds). Genetic Diversity in Plants .p. 21-44.Plenum, New York.

- Golembiewski, R.C., Danneberger, T.K. and Sweeney, P.M.1997. Potential of RAPD Markers for Use in the Identification of Creeping Bentgrass Cultivars. *Crop Sci,* **37:** 212-214.

- Gregor, D., Hartmann, W. and Stosser, R. 1994. Cultivar Identification in Prunus Domestica using Random Amplified Polymorphic DNA Markers. *Acta Horticulturae,* **359:** 33-41.

- Guirgis, A.A., Fayed, A.H., Abd El-Sayyed, S.M. and Ismail, M.A.1990. Nature of Genetic Variability and Association Analysis in Okra Population. *Zagazig. J. Agri. Res,* **17**(4A): 117-118.

- Hamon, S., Charrier, A. and Koechlin, J.1990. Potential Contribution to Okra Breeding through the Study of their Genetic Resources. Report of an international Workshop on Okra Genetic Resources Held at the National Bureau for Plant Genetic Resources, New Delhi, India, **8-12** October, 77-88.

- Hamon, S., 1990. Potential and Limitations of Standardized Discriptor for the Genus ***Abelmoschus*** (Okra). Report of an international

Workshop on Okra Genetic Resources Held at the National Bureau for Plant Genetic Resources, New Delhi, India, **8-12** October, 48-50.

- Helmy, E. M. 1991. Variability and Improved Inbreds Derived from Eskandarani Cultivars of Okra ***(Abelmoschus esculentus L.).*** *Alex.J. Agric*, **36**(2): 110-128.

- Hindagala, C.B., Jayawardena, S.D.G., Siriwardena, K.P.D., and Liyanage, A.S. 1990.Report of an international Workshop on Okra Genetics Resources Held at the National Bureau for Plant Genetic Resources., New Delhi, India, **8-12** October, 15-20.

- Kalia, H. R. and Padda, D. S. 1962. The Inheritance of some Fruit Characters in Okra. *Indian J. Gen. Pla. Bre*, **22**(3): 248-251.

- Karihaloo, J.L., Brauner, S. and Gottlieb, L.D.1995. Random Amplified Polymorphic DNA Variation in the Eggplant, ***Solanum melongena L.*** (Solanaceae). *Theor. Appl. Genet* , **90:** 767-770.

- Ketsa, S. and Chutichudet, B. 1994. Pod Growth Development, Biochemical Changes and Maturity Indices of Okra CV.Ok#2. *Acta Horticultrae*, **369**: 368-378.

- Kaul, T., Lal, G., and Peter,V.K.1978. Correlation and Path–Coefficient Analysis of Components of Earliness, Pod Yield and Seed Yield in Okra. *Indian J. Agric. Sci*, **48**(8): 459-463.

- Klein–Lankhors, R.M., Vermunt, A., Welde, R., Liharska,T., and Zabel, P.1991. Isolation of Molecular Markers for Tomato (***Lycopersicon esculentum L.***) using Random Amplified Polymorphic DNA (RAPD). *Theor. Appl. Genet*, **83**: 108-114.

- Kuckuck, H., Kobabe, G. and Wenzel,G.1991. *Fundamental of Plant Breeding, First. Edition*, Springerverlag Berlin Heidelberg. pp: 3-5

- Kumar, P.P., Yau, J.C.K. and Goh, C J. 1998.Genetic Analysis of Heliconia Species and Cultivars with Randomly Amplified Polymorphic DNA (RAPD) Markers. *J. Amer. Soc. Hort. Sci*, **123**(1): 91-97.

- Marsh, L. and Jones, R. 1990. Growth of Okra and Fruiting Pattern as Affected by Growth Regulators. *Hortscience*, **25** (4): 431-432.

- Martin, F.W., Rhodes, A.M., ortiz, M. and Diaz ,F.1981. Variation in Okra. *Euphytica*, **30:** 697 –705.

- Mishra, R. S. and Chhonkar, V. S. 1979. Genetic Divergence in Okra. *Indian J. Agri. Sci*, **49**(9): 247-249.

- Moreno, S., Gogorcena, Y. and Ortiz, J.M. 1995. The Use of RAPD Markers for Identification of Cultivated Grapevine *(Vitis vinifera L.). Scientia Horticulturae* ,**62:** 237-243.

- Nath, P., and Dutta, O.P. 1970. Inheritance of Fruit Hairness, Fruits Shin Colour and Leaf Lobing in Okra, (*Abelmoschus esculentus L.) Can. J. Genet. Cytol.* **12:** 589-593.

- Newton, C.R and Graham, A. 1994. *PCR.* Bios scientific Publisher Ltd, PP: 2-7.

- Raji, J. A. 1994. Field and Glass-House Screening of Okra ***(Hibischus esculenuts L.)*** Genotypes for Yield and Intercropping. *Indian. J. Agri. Sci.* **64** (7): 454-458.

- Rana, R. S and Thomas, T. A. 1990. Plant Genetic Resources Activities in Okra and Indian Perspective. Report of an International Workshop on Okra Genetic Resources Held at the National Burea for Plant Genetic Resources, New Delhi, India, **8-12** October, 38-47.

- Rao, S. T. and Sathyavathi, G. P. 1977. Genetic and Environmental Variability in Okra. *Indian J. Agri. Sci*, **47**(2): 80-81.

- Rychlik, W., Spencer, W.J. and Rhoads, R.E.1990. Optimization of the Annealing Temperature for DNA Amplification *in Vitro. Nucleic Acids Research,* **18**(21): 6409-6412.

- Saiki, R.K., Gelfand, D. H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G. T., Mullus, K.B. and Erlich, H.A. 1988. Primer-Directed Enzymatic Amplification of DNA with a Thermostable DNA Polymerase. *Science*. **239**: 487-490.

- Salameh, N.M. 1998. Studies on the Inheritance of some Fruits Characters of okra (***Hibiscus esculentusL.***), 9-10. MSc. Thesis, University of Jordan, Amman, Jordan.

- Sharma, B. R. and Mahajan, Y. P. 1978. Line X Tester Analysis of Combining- Ability and Heterosis for some Economic Characters in Okra. *Scientia Horticulturae*, 9: 111-118.

- Sambrook, J., Fritsch, E.F. and Maniatis, T. 1989. Molecular Cloning: a Laboratotry Manual. *Second. Edition* Cold Spring Harbor Laboratory Press.

- Singh, S.P., and Singh,H.N.1979. Hybrid Vigour for Yield and Its Components in Okra .*Indian J. Agric.Sci*, 49(8) : 596 –601.

- Singh, S.P., Srivastava, J.P. Singh, H.N. and Singh, N.P. 1977. Genetic Divergence and Nature of Heterosis in Okra. *Indian. J. Agri. Sci,* 47(1): 546-551.

- Sosinski, B. and Douches, D.S.1996. Using Polymerase Chain Reaction-based DNA Amplification to Fingerprint North American Potato Cultivars. *Hort Science*, 31(1): 130-133.

- Steel, R.G.D., and Torrie, J.H. 1980.*Principles and Procedures Statistics Abiometrical Approach, Second Edition*. McGraw-Hill Co. INC. 633 pp.

- Tivang, J., Skroch, P.W. and Nienhuis, J. 1996. Randomly Amplified Polymorphic DNA (RAPD) Variation Among and Within

Artichoke *(Cynara scolymus L.)* Cultivars and Breeding Populations. *J. Amer. Soc. Hort. Sci*, **121**(5): 783-788.

- Vazquez, F.J., Jimenez, J.M.C. and Vico, F.R. 1996. RAPD Fingerprinting of Pepper *(Capsicum annuum L.)* Breeding Lines. *Capsicum and Egg plant Newsletter*, **15:** 37-40.

- Velayudhan, K.C. and Upadhyay, M.P. 1994. Collecting Okra, Eggplant and their Wild Relatives in Nepal. *Plant Genetic Resources Newsletter*, **97**: 55-57.

- Weising, K., Nybom, H., Wolff, K. and Meyer, W. 1994. *DNA Fingerprinting in Plants and Fungi.*, BocaRaton Ann Arbor London Tokyo. PP: 23-25.

- Williams, C.E. and Ronald, P.C. 1994. PCR Template–DNA Isolated Quickly from Monocot and Dicot Leaves Without Tissue Homogenization. *Nucleic Acids Research,* **22** (10): 1917 – 1918.

8- Appendix (A)

- Laboratory Materials.

1- Chemicals.

Chemicals used for isolating DNA from okra and DNA electrophoresis

	Chemical name	Formula	Formula Weight	Country	Company	Catalog Number
1	Agarose (for field electrophoresis running gel)			U.S.A.	Sigma	A-2929
2	Boric acid	BH_3O_3	61.83	U.S.A.	Fluka Biochemika	15663
3	Bromphenolblue (3,3",5',05"-= tetrabromo phenol sulfonephthalein)	$C_{19}HgBr_4$ O_5 SNA	691.9	U.S.A.	Sigma	B-5525
4	Chloroform	$CHCl_3$	119.38	U.S.A.	Sigma	C5312
5	Ethanol	C_2H_6O	46.07	Germany	PTZ	109/21
6	Ethidium bromide	$C_{21}H_2ON_3Br$	394.3	U.S.A.	Sigma	E-8751
7	Ethylendiamine tetra acetic acid (EDTA)	$C_{10}H_{16}N_2O_6$	292.2	U.S.A.	Sigma	E-6758
8	Hexadcyltrimethyl ammoniumbromide (CTAB)	$C_{19}H_{42}NBr$	364.5	U.S.A.	Sigma	H-5882
9	2-Hydroxyethyl mercaptan, B-mercaptoethanol, or 2-merca-ptoethonol	$C_2H_6O_5$	78.13	U.S.A.	Sigma	M-6250
10	Isomaylalcohol (isopentyladohol, 3 methyl 1-butanol)	$C_5H_{12}O$	88.15	U.S.A.	Sigma	I-3643
11	Isopropylalcohol	C_3H_8O	60.1	U.S.A.	Sigma	I-0398
12	Nitrogen (liquid- 196 C°)	N	14	Jordan	National gas company	-
13	Polyvinylpolypyrrolidone	Insoluble	High molecular weight	U.S.A.	Sigma	P-6755
14	Sodium chloride	NaCl	58.44	U.S.A.	Sigma	S-7653
15	Sucrose	$C_{12}H_{22}O_{11}$	3 42-3	U.S.A.	Sigma	5-0389
16	(Tris(hydroxy methyl) amino-methane	$C_4H_{11}NO_3$	121.1	U.S.A.	Sigma	T-679

2- Instruments.

2-1 Disposable Materials.

	Name	Country	Company	Describtion	Uses
1.	Aluminum foil	Jordan – Sahab	Leena company	5 m X 45 cm	To cover material in order to autocalve
2.	Gloves		Medical company	Latix	To wear on hand and protect it
3.	Hygenic tissue	Jordan	Sahab –company	Papper	To clean tools
4.	Mouth mask	Jordan	Medical company		To cover mouth and keep it from inhalin toxic substance
5.	Microfugetubes	Germany	Eppendorf	1.5 ml with cover	DNA extraction and storage
6.	PCR Tubes	Germany	Eppendorf	0.2 ml with cover	For PCR use
7.	Pipett tips -Blue -White -Yellow	 Germany Germany Germany	 Eppendorf Eppendorf Eppendorf	 (100-1000 µl) (0.5–10 µl) (2–200 µl)	 For pipetting solutions For pipetting solutions For pipetting solutions

2-2 PCR and Equipment for DNA Isolation.

	Name	Country	Company	Description	Uses
1.	Autoclave			Heated tools at 121C° and Pressure 15 bar	Sterilization
2.	Centrifuge	Germany	Eppendorf	5415 c [14000 rpm min^{-1}]	Precipitate nucleic acids and chloroform extraction
3.	Face mask	U.S.A.	U.S. safety ANSI 287	Specific for U.V.	To protect face from U.V. light
4.	Microwave oven	U.S.A.	Frigidare		To melt agarose for prepare gel
5.	Adjustable pipettes	Germany	Eppendorf	- 0.5-10 µl (44110000.018) - 2.0-20 µl (491 0000.034) - 10 -100 µl (491 0 000.042) - 50 – 200 µl (4 910 000.093) - 100 – 1000 ml (4910000.069)	To get accurate volume for each tip in DNA isolation and PCR master mix
6.	PH meter	Germany	Bedien-ungsanleitung	—	Measure pH of solution
7.	Racks	U.S.A.	Nale Gene cryoware and sybaron 8-403-970595	Holes with different colors	To hold tubes of samples
8.	Frezer (-20 C°)	U.S.A.	Kelvinator	UC 76F-7 11500	Keep the samples until used
9.	Revolving hot plate	Great Britain	LTE Scie-Ntrific	—	Preparation stock solution
10.	Sensitive balance	Germany	Sartorius	BP 3105	Weight plant tissues and chemicals
11.	Screw driver	Germany	Felo	23650	To grinding the tissues
12.	Thermocycler	U.S.A.	Gene AMP system 9600	Programmable Perkin Elmer Ltd- England	DNA amplification for PCR
13.	Vortex	U.S.A.	Scientific industries INC	Vortex Gene2 model G560 & G 560E	Homogeniz the samples
14.	Waterbath	Germany	Julabo	SW 20 / 4	To fixed temperature for different uses

2-3 Electrophoresis Equipment.

	Name	Country	Company	Description	Uses
1.	Power supply	Beligum	BiotecFischet	Microcomputer electrophoresis power supply PHERO-STAB 330	Provide electric charge to electrophoresis buffer to separate DNA
2.	Submarine macrogel	U.S.A.	Sigma	-Length 31 cm from anode to cathode -Comb length 19 cm -Has 2 combs with 22 holes -Length 18 cm	DNA electrophoresis for large number of samples
3.	Submarin minigel	U.S.A.	Sigma	-Comb length 8.6 cm -Has two type of combes 8 holes and 12 holes	DNA electrophoresis for few samples

2-4 PCR Product Photos.

	Name	Country	Company	Description	Uses
1.	Camera	U.S.A.	Polaroid	MP4^{+}	Taking photo for DNA profile and documented
2.	Instant Films		Polaroid	667(positive) 3 ¼ X 4 ¼	
3.	Ultra Violet light source (U.V.)	Germany	Faust Conrude Benda 410796	254 nm and 366 nm wave-length	Transilluminator for DNA gels

3- Amplification Components used in RAPD.

Name	Country	Company	Description
1- PCR 10 X buffer	U.S.A.	Sigma	See Appendix B-3
		Perkin Elmer	
2- Nucleotides	U.S.A.		2'- deoxyadenosine 5'- triphosphate sodium salts)
100 mM dATP			2'- deoxycytidine 5'- triphosphate sodium salts).
100 mM dCTP		Promega	2'-deoxy guanosine 5'-triphosphate sodium salts)
100 mM dGTP			2'-deoxy thymine 5'- triphosphate sodium salts)
100 mM dTTP			catalog # U1330, Madison
3-PCR size marker (50-2000 bp)	U.S.A.	Sigma	Catalog No. P-9577
4- Random Primers	U.S.A.	Operon technologies	See Table 4
5-*Taq* polymerase	U.S.A.	Sigma	See Appendix B-II
		Perkin Elmer	Catalog No. N 808-0160
		Promega	Catalog No. M-1861

Appendix B

1-Stock Solutions and Preparation.

I–Buffers.

1- Extraction Buffer (CTAB).

1-1 2 X CTAB buffer : 1.4 M NaCl ,100 mM Tris Base pH 8.00, 2% (hexadecyl trimethylammonium bromide) and 20 mM EDTA.

This was prepared as following: 100 ml (1M Tris pH 8.0)+ 20 gm CTAB + 80 ml (0.25 M EDTA) + 81.8 g NaCl and completed to 1L with distelled water

Note: 2-mercaptoethanol and PVPP were added just before using the extraction buffer [20 ml of 1.33 X CTAB buffer, 200 µl of 2- mercaptoethanol and 80 mg PVPP]

1-2 1.33 X CTAB buffer: 200 ml of 2X CTAB buffer diluted with 100ml distilled water.

2- TE Buffer for Dissolving DNA Pellets.

- 10.0 mM Tris Base of (pH 7.4)
- 1 mM EDTA (pH 8.0) These prepared as following: 5 ml (1 M Tris), 2 ml (0.25 M EDTA) and distilled water was added to 500 ml final volume.

3- 10 X PCR Buffer.

- 100 mM Trizma. HCl, pH 8.3 at 25 C^{o}.
- 25 mM $MgCl_2$,
- 500 mM KCL
- 0.01 % (W/V) gelatin

4-10 X TNE Buffer.

- 100 mM Tris base
- 10 mM EDTA
- 2 M NaCl
- pH: 7.4

5- TBE Running Buffer. Tris-Borate-EDTA.

5-1 Component of 5 X TBE to prepare 2 l.

- 108 gm Tris base
- 55 gm boric acid
- 80 ml from (0.25 M EDTA)

5-2 0.5 X TBE working running buffer

- 1800 ml distilled water added to 200 ml of 5 X TBE buffer.

6- EDTA Buffer (0.25 M).

- 146.1 gm (EDTA) /2 litter
- 40 gm NaOH /2 litter distilled water (1M NaOH)
- pH = 8.00

7- 1 M HCL

- 8.6 ml HCL /100 ml distilled water

8- Chloroform / Isoamyl alcohol 24:1.

- 96 ml chloroform
- 4 ml isoamyl alcohol

9-Gel Loading Buffer (6X).

- 0.25 % bromophenol blue
- 40 % (W/V) sucrose
- 1ml (1M tris buffer pH 8.0)

Prepared as following: 50 mg (bromophenol blue)+ 8 gm (sucrose) dissolved in 20 ml distilled water and 1ml of tris buffer.

10- Ethidium Bromide Solution (10mg/ ml).

1.0 gm ethidium bromide

100 ml distilled water

Caution: Ethidium bromide is very highly mutagentic and carcinogenic and should be kept in dark bottle covered with aluminum foil

11- 70% Ethanol.

- 70 % Absolute ethanol
- 30 % distilled water

II ENZYMES.

- Extraction enzyme (Ribonuclease A)

  RNase (10 mg/ml) free of DNase (Pancreatic Bovine): 10 mg of RNase dissolved in 1 ml (TE) pH 7.4 and stored at -20C°.

- *Taq* polymerase

  The enzyme is sold in 500mM Tris-HCl (pH 8.0 at 25C°), 100 mM NaCl, 0.1mM EDTA, 1mM DTT, 50% glycerol, 0.5% Tween® 20 and 0.5% Nonidiet® P40.

Appendix C: Mean squares for morphological traits in nineteen okra landraces collected from different regions in Jordan and control evaluated in the field in 1998.

Source	df	Days to flowering (days)	Plant height at flowering (cm)	Final plant height (cm)	Immature pod width (cm)	Immature pod length (cm)	Mature pod length (cm)	Immature pod weight (g)	Pods per plant	Pod yield / plant (g)	Seeds per pod
Blocks	2	897.98	359.15	3973.11	0.013	1.55	124.26	4.70	309.86	8971.64	4145.33
Landraces	19	** 655.81	** 1623.17	** 5601.53	** 0.389	** 1.89	** 96.40	** 9.30	** 252.81	** 2926.09	** 1084.18
Experimental error (Blocks X Landraces)	38	^{NS} 110.52	^{NS} 270.02	**2526.28	^{NS} 0.025	^{NS} 0.84	^{NS} 19.164	^{NS} 1.68	^{NS} 145.82	**2799.79	**645.56
Sampling error (among plants within landraces)	1140	53.043	100.64	324.83	0.010	0.446	6.255	0.82	54.04	889.71	239.78
Total	1199										

** Significant at p= 0.01, NS: non significant.

19. Microlitter	µl
20. Milligram	mg
21. Millimolar	mM
22. Nanogram	ng
23. National Center of Agricultural Research and Technology Transfer	NCARTT
24. Operon	OP
25. Pages	PP.
26. Per	/
27. Percent	%
28. Polymerase Chain Reaction	PCR
29. Ribonucleic Acid	RNA
30. Random Amplified Polymorphic DNA	RAPD
31. Randomized Complete Block Design	RCBD
32. Rotation per minute	rpm
33. Standard Deviation	SD
34. DNA polymerase isolated from *Thermus aquaticus*	*Taq*
35. Solution of Tris- EDTA (PH = 8.0)	TE
36. Volt	V
37. Weight / Volume	W/V

الملخص

التباين الوراثي بين و داخل سلالات الباميا المحلية في الأردن

إعداد

إبراهيم محمد الرواشدة

إشراف

الأستاذ الدكتور محمود قصراوي

مشرف مشارك

د. سمير مسعود

تم تقدير التباين الوراثي حقلياً بين وداخل تسع عشرة سلالة باميه محلية بالإضافة إلى صنف تجاري واحد حقلياً إعتماداً على الاختلافات الظاهرية. كما استخدم خمس سلالات محلية وصنف تجاري واحد لتحديد التباين الوراثي بين المدخلات على مستوى الحمض النووي الرايبوزي الخالي من الأكسجين DNA باستخدام تقنية تحليل واسمات DNA العشوائية المضخمة متعددة الأشكال (RAPD). ضمت الدراسة الصفات البستانية والثمرية التالية: عدد الأيام حتى الازهار، طول النبات عند الازهار، طول النبات في نهاية موسم النمو، عرض القرن الأخضر (غير ناضج) لون القرن الأخضر (غير ناضج)، طول القرن الناضج، وزن القرن الأخضر (غير ناضج)، عدد القرون لكل نبات، إنتاجية القرن لكل نبات، عدد البذور لكل قرن، لون الساق، لون الورقة وحاملها ولون القرن.

التحليل الاحصائي باستخدام المدى، والانحراف المعياري ومعاملات التباين أظهرت فروقاً معنوية بين وداخل معظم المدخلات. عدد الثمار لكل نبات سجل أعلى تباين مظهري 50.2% وعدد الأيام حتى الازهار أقل تباين مظهري 8.3%، حيث تبين قابليتها للاختيار.

الصفات المدروسة أظهرت مدى واسع من الاختلافات مما يدل على أنها صفات وراثية فقط. بعض سلالات الباميه المحلية أظهرت ارتباط بين لون الساق، لون القرن، ولون الورقة مما يدل على أن هناك جينات مشتركة تتحكم بهذه الصفات. أما عدد الأيام حتى الازهار، انتاجية القرون لكل نبات، عرض القرن الأخضر ولون القرن ممكن أن تستخدم للتمييز بين المدخلات. المعلومات المستمدة من استخدام بادئات عشرية أشارت لإمكانية استخدام تقنية الـ (RAPD) في كشف الاختلافات الوراثية بين وداخل السلالات المحلية. استخدام بادئة OPA13 كان ذو فاعلية في تضخيم حزم الـ DNA داخل سلالات الباميا المحلية، فإنه قد يكون بالامكان تميز سلالة محلية واحدة باستخدام وجود أو عدم وجود قطعة الـ DNA ذات الحجم 386 bp أو 1000 bp باستخدام هذه البادئة. إن التحليل بواسطة استخدام تقنية الـ RAPD قد يكون فعال وذو قيمة عالية لكشف الاختلافات بين وداخل السلالات المحلية أو الصنف. على الرغم من أن التباينات المظهرية العالية التي أظهرتها سلالات الباميا المحلية، فإن التباينات على مستوى الحمض النووي الرايبوزي لم تكن كافية للحصول على خصائص معرفة لكل سلالة أو صنف، إن تحليل بادئات عشوائية إضافية في تقنية الـ RAPD يمكن أن يعرف بادئات مميزة ممكن استخدامها في برامج التربية.

هذه الدراسة بدأت بالوصف الجزيئي لسلاسلات الباميا المحلية المجمعة لمختلف المصادر الوراثية وتعتبر الأساس لبرامج التحسين.