Yield and AFLP Analyses of Inter-Landrace Variability in Okra (*Abelmoschus esculentus* L.)

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Abstract: Evaluation of genetic diversity within landrace collections is important to plant breeders who desire sources of genes for particular traits. Twenty one landraces of okra (*Abelmoschus esculentus* L.) collected in Jordan and one imported cultivar "*Clemson spinless*" were evaluated for yield and amplified fragment length polymorphism (AFLP) markers in an attempt to differentiate among the landraces. Fruit number and fruit yield per plant were evaluated in 2011/2012 growing season. These two yield descriptors, in addition to previously reported data of spine existence and fruit color were analyzed by Ward's cluster analysis. The 22 germplasms were grouped into three clusters of phenotypic diversity. There was some correspondence between the geographic collection sites of landraces and their inclusion in particular clusters. The AFLP primer combination with the highest polymorphic information content (PIC) and marker index (MI) values was *MseI*-CAA/*EcoRI*-AAG, indicating that it had the most discriminatory power among the eight tested primer combinations. Using the generated AFLP data, the un-weighted pair group method with arithmetic average (UPGMA) ordered germplasms into three groups based on Dice similarity coefficient. Although results obtained from UPGMA were similar to the ones obtained by principal coordinate analysis (PCA), the correlation between distance coefficients based on phenotypic and AFLP markers was not significant. Nevertheless, both analyses were useful for identifying likely duplicates within the collection, and will facilitate identification of potentially different sources.
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1.Introduction

Okra *Abelmoschus esculentus* L. (Moench) is a commercially important vegetable crop in tropical and subtropical regions of the world (Hammon and Van Sloten, 1989) especially Afghanistan, Turkey, Yugoslavia, Western Africa, and Brazil. It has a prominent position among fruit vegetables due to its multiple virtues like high nutritive and medicinal value, ease of cultivation, wide adaptability, and pleasant flavor (Lamont, 1999; Reddy *et al.,* 2012). In Jordan, okra is planted over an area of 10,428.3 dunums with average production of about 6814 tons (Department of General Statistics, 2010).

Genetic variability based on phenotypic traits had been studied by several studies (Gill *et al.,* 1997; Ghai *et al.,* 2005; Hussain *et al.,* 2005; Kamari and Chaudhury, 2006; Bello *et al.,* 2006); Sing *et al.,* 2007). For example, Hussain *et al.,* (2005) found a high genetic variability for fruit yield, seed number, fruit number and node number. Also, El-Taher (1990) recorded a high genetic variability among and within landraces for different morpho-agronomic characters for okra landraces collected from Sudan.

Although the genetic base of okra can be rated as high diversity (Saifullah *et al.,* 2010), it is still difficult to distinguish genotypes based on their external morphology alone. Assessment of genetic diversity based on phenotype does not reliably reflect the genetic variation. Molecular tools are more trustworthy, independent from the environmental conditions and show higher levels of polymorphism. Tanksley (1983) listed five properties that distinguish molecular markers from morphological markers. These properties are: genotypes can be determined at the whole plant, tissue and/or cellular level, a relatively large number of naturally occurring alleles exist at many loci, phenotypic neutrality, alleles at many loci are codominant, thus all possible genotypes can be distinguished, and few epistatic or pleiotropic effects are observed.

Comparing to other crops, okra information at the DNA level has lagged behind, with few studies on DNA markers applications. Using randomly amplified polymorphic DNA (RAPD) technique, Rawashdeh (1999) collected 19 local landraces of okra (*Abelmoschus esculentus*) from Jordan and reported significant differnces of their yield and genetic makeup. Amplified fragment length polymorphism (AFLP) is a method that has become widely applied in plant population genetics since its development in 1995 (Vos, *et al.,* 1995). Compared to other methods, the major advantages of AFLP lie

in its high diversity index and the number of amplified loci per assay unit (Russell, *et al.,* 1997). The AFLP markers allow polymerase chain reaction (PCR) amplification of restricted fragments (Akash, 2011). To our knowledge, no previous studies were employed to get benefit from the advantage of AFLP markers to investigate okra at the DNA level. The objectives of this study were to assess the genetic distinctiveness in 21 okra landraces plus one control commercial cultivar commonly planted in Jordan environment using AFLP markers.

2.Materials and methods

Yield analysis

A total of 21 seed samples of okra landraces were obtained from National Center for Agricultural Research and Extention (NCARE) Amman-Jordan (Table 1). Six Jordanian provinces were scaned to obtain these 21 landraces (Table 1). Also, a commercial check cultivar *(Clemson spinless)* was added to our experiment.

Seeds of the 21 okra landraces and the commercial cultivar were sown on June, 5, 2011. A drip irrigation system with black plastic mulch was used. Seeds were sown with 0.6 m between rows and 0.4 m between plants. Each landrace occupied a single row of 1.2 m long. To strengthen vegetative growth, at early stage, Ammonia was added while Wafer fertilizer (2 g/plant; 20:20:20 NPK) was added 30 days after planting. Wettable sulphur (2 g/liter) was added to protect plants against powdery mildew disease, and Evisect (20 ml/liter) was added to protect plants against whitefly. Fruit number and fruit yield per plant were measured when fruits reached the marketing stage as immature fruits.

Yield data analysis

Three blockes of the 21 okra landraces and the commercial cultivar were statistically used in a randomized complete block design (RCBD). The RCBD was performed using Statistical Analysis System (SAS) software (SAS, 2002) using mixed procedure and yield means were separated using Fisher's protected Least Significant Difference (LSD) at propability level (P=0.05). Fruit number and fruit yield per plant in addition to previously reported data of their spine existence and fruit color were used as input matrix for cluster analysis based on ward's method (Ward, 1963) using SAS software (SAS, 2002).

AFLP analysis

Four to five young and healthy leaves were collected from each germplasm (21 landraces and commercial cultivar) and bulked together for DNA isolation. DNA was extracted according to the cetyl trimethyl ammonium bromide (CTAB) protocol (Torres, *et al.,*1993) with minor modifications as described by Abu-Amer, *et al.* (2011). Samples DNA were quantified through comparing the size and intensity of each sample band with 100 bp DNA mass ladder (Promega; Madison, USA) using agarose gel (0.7%).

AFLP data were generated using eight different selective primer combinations (Table 2). The generation of the data was performed according to Vos, *et al.* (1995) with some modifications as mentioned by Akash and Kang (2010).

AFLP data analysis

Bands of the same size in different samples were treated as the same locus, and scored as present (1) or absent (0) in each germplasm. Loci were considered polymorphic if present in at least one germplasm and absent in the others and vice versa. For each AFLP primer combination, the polymorphic information content (PIC) and marker index (MI) were calculated as described by De Riek *et al.,* (2001) and Varshney, *et al.* (2007), respectively. Genetic similarities were estimated based on Dice coefficient option from the NTSYS-pc software package (Rohlf, 1998). Cluster analysis was constructed based on the unweighted paired-group method using arithmetic averages (UPGMA). A principal coordinate analysis (PCO) was also performed using the Eigen functions of NTSYS-pc to assess genetic relationships. The first and second PCO scores were plotted for visualization.

3.Results and discussions

Yield data analysis

The fruit yield per plant is based on number of fruits per plant and weight in gram per plant (Dewdar *et al.,* 1987). Analysis of variance indicated highly significant differences among okra landraces for fruit yield per plant with $p \leq 0.001$. The overall average yield per plant was 75.73 g that ranged from 31.5 to 135 g. Mean separation showed significant differences among local landraces and the control cultivar, Jo161 gave significantly the highest fruit yield (134.09 g), while jo894 landrace gave the lowest fruit yield (31.5 g) (Table 1). These results found to agree with those reported by Rawashdeh (1999) who found that landraces varied significantly in fruit yield per plant and he reported that the fruit yield per plant ranging from 7 to 230 g in local landraces. Sonia (1999) observed that marketable yield per plant varied from 154 to 467g among different okra genotypes. Fruit yield was influenced mainly by the number of pods per plant (Kaul *et al.,* 1978).

Reddy *et al.*, (2012) revealed a considerable genetic diversity among 100 genotypes of okra for all

17 quantitative characters which pertaining to the growth, earliness and yield. Katung (2007) studied two okra varieties; he found significant differences for fruit yield among varieties. Saifullah and Rabbani (2009) reported that the high heritability estimates along with considerable genetic advance were noticed in fruit yield per plant. Nwangburuka *et al.* (2011) found a wide range of diversity among 29 okra accessions sourced from different agro-ecological regions in Nigeria for fruit yield.

Our results showed that there were no significant differences in number of fruit per plant between landraces with $P > 0.05$, but it showed a significant difference between landraces and the control cultivar *Clemson spinless* except for jo887. The control cultivar *Clemson spinless* had significantly the highest number of fruit per plant (29), while the 21 landraces gave an average of 18 fruits per plant. Number of fruits per plant showed significant differences among landraces ranging from 11 – 19 fruits per plant (Rawashdeh, 1999). Ariyo (1990) found that the number of fruits per plant showed low heritability estimate which supports the idea that environmental factors have a considerable effects on such important. These variabilities in number of fruits and yield per plant are the selection basis for development of new variety of okra

AFLP data analysis

In the last two decades, AFLP has become one of the most widely used molecular markers to study genetic structure and relationship (Foll, 2010). In our study, eight AFLP primer combinations were used to profile the 21 okra (*Abelmoschus esculentus*) landraces and the control cultivar (*Clemson spinless*) (Figure 1). As shown in table 4, a total of 722 loci were generated using the eight primer combinations. Out of those, 227 (31.8%) polymorphic loci ranging in size from 54 to 645 bp were scored. The number of amplified loci per primer ranged from 72 loci (*MseI*-CAT and *EcoRI*-AGG) to 109 loci (*MseI*-CAC and *EcoRI*-ACC) and the polymorphism percentage ranged from 26.8% (*MseI*-CAG and *EcoRI*-AAC) to 41% (*MseI*-CAG and *EcoRI*-ACT). The number of polymorphic loci per primer ranged from 21 loci (*MseI*-CAT and *EcoRI*-AGG) to 34 loci (*MseI*-CTG and *EcoRI*-AAG) with an average of 28 loci. The PIC values ranged from 0.23 to 0.31 with an average of 0.26 and the MI values ranged from 5.95 to 9.25 with an average of 7.5. The primer combination with the highest PIC and MI values was *MseI*-CAA/*EcoRI*-AAG, indicating that it had the most discriminatory power among the eight tested primer combinations (Table 4).

The relatively high polymorphism percentage (31.6%) was sufficient to estimate genetic variation among the 21 studied okra landraces and the control cultivar (*Clemson spinless*). A higher polymorphism percentage of about 50% was obtained when 16 AFLP primer combinations were used to investigate genetic similarities among 21 cotton genotypes (Adawy *et al.,* 2005).

Cluster and Principal Coordinate Analyses:

Dice similarity coefficient (Dice, 1945) which is a matching coefficient for binary data generated from AFLP analysis, was used to cluster the 21 okra (*Abelmoschus esculentus*) landraces and the control cultivar (*Clemson spinless*) with the unweighted pair group method with arithmetic average (UPGMA). The 22 genotypes fell into three distinct clusters plus seven weekly grouped landraces (Figure 2). All landraces in cluster I came from Irbid province except for one landrace that came from different province. Cluster I also included the introduced commercial cultivar. The similarity level among the first cluster ranged from 0.77 to 0.88. The highest genetic similarity percentage (0.88) was observed between jo155 and jo158, followed by 0.83 between the control commercial cultivar (*Clemson spinless*) and jo903. Cluster II and cluster III consisted of landraces from different provinces. In the second cluster that consists of four landraces, the highest similarity coefficient (0.82) was observed between jo882 and jo885, while the lowest similarity coefficient (0.78) was observed between jo887 and the rest of cluster II landraces. In cluster III, jo898 showed the highest similarity with jo900 landrace (0.88). The Lack of concordance between UPGMA groups and provinces had been reported inokra by Reddy et al. (2012). However, the relative high similarity among landraces was expected because it is a self pollinated species (Hamon and Koechlin 1991). The first two dimensions of the principal coordinate analysis explained 77.6% of the total variation. The first and second dimensions explained 74.2% and 3.4%, respectively. The groups obtained from PCO and UPGMA cluster analyses were similar. For example, jo155, jo158, and jo903 were grouped with the control cultivar in both analyses (Figure 3).

Ward's based clustering based on phenotypic data showed also three main clusters (cluster I: *Clemson spinless*, Jo161, Jo877, Jo883, Jo885, J893, and J900; cluster II: Jo158, Jo875, and Jo894; Cluster III: rest of landraces. These results showed that small but significant variation exists among landraces and this may be due to seed exchange between farmers and natural cross between landraces and cultivars which represent the highest source of okra for our farmers in Jordan. Gulsen *et al*., (2007) showed that dendrogram analysis based on phenotypic marker data was successful in distinguishing 23 Turkish okra

(*Abelmoschus esculentus* (L) Moench) genotypes. The three Ward's clusters were weakly consistent with the three larger groups obtained from clustering based on AFLP markers. Our results indicate a lack of significant correlation between phenotypic as inferred by Ward's clustering and actual AFLP genetic profile as inferred by UPGMA clustering. This lack of correlation suggests the lack of genetic linkage between AFLP loci and studied phenotypes.

One of the fundamentals of plant breeding is the presence of genetic diversity (Ariyo, 1993), also it is considered as an aspect of successful naturally-selected traits among different environmental conditions. Estimation of genetic diversity provides an essential lead for obstacles that may arise in any stage of plant breeding program (Dudley and Moll, 1969). In fact, presence of genetic diversity in any base population is a crucial necessity for the success of any breeding programs (Bangaru *et al*., 1983).

Table 1. List of 21 okra (*Abelmoschus esculentus* L.) landraces and one control cultivar with their collection province fruit color and spine existence.

Landrace	Province	Location	Fruit color	Spine existence
Jo155	Madaba	Mansorah	Green with red	Absent
Jo158	Irbid	Husun Camp	Green with red	Present
Jo161	Irbid	Maru	Green	Absent
Jo578	Jerash	Rashaydeh	Green	Present
Jo875	Irbid	Fo'arah	Green with red	Present
Jo877	Madaba	near Nadeem hospital	Green	Absent
Jo880	Zarqa	Sukhneh	Green with red	Absent
Jo881	Irbid	Hakama	Green with red	Present
Jo882	Irbid	Hakama	Green with red	Present
Jo883	Irbid	Samma	Green	Present
Jo885	Irbid	Al-Barha	Green	Present
Jo887	Madaba	Mansorah	Green with red	Absent
Jo891	Irbid	Sama Al-Rosan	Green	Absent
Jo892	Irbid	Fo'arah	Green	Present
Jo893	Madaba	Faisaleyeh	Green with red	Absent
Jo894	Madaba	Mansorah	Green	Absent
Jo895	Mafraq	Saba Al-sir	Green	Present
Jo898	Aqaba	Gwerah	Green with red	Absent
Jo900	Madaba	Ghernata	Green	Absent
Jo902	Mafraq	Saba Al-sir	Green	Present
Jo903	Irbid	Hakama	Green	Present
Clemson spinless (Commercial cultivar)			Green	Absent

Table 2. Adapters and primers used for ligation, pre-amplification and selective amplification steps of the AFLP procedure.

Type	Sequence (5'-3')	
	*Eco*RI	*Mse*I
Adapters	CTCGTAGACTGCGTACC AATTGGTACGCAGTC	GACGATGAGTCCTGAG TACTCAGGACTCAT
Pre amplification primers	GACTGCGTACCAATTCA	GATGAGTCCTGAGTAAC
Selective amplification primers	GACTGCGTACCAATTCAAAG GACTGCGTACCAATTCAAGG GACTGCGTACCAATTCAACC	GATGAGTCCTGAGTAACCAT GATGAGTCCTGAGTAACCTG GATGAGTCCTGAGTAACCAA GATGAGTCCTGAGTAACCAC

Table 3. Results of mean separation for fruit yield per plant of the 21 okra (*Abelmoschus esculentus*) landraces and the control cultivar (*Clemson spinless*).

Landraces	**Fruit yield (g)**
Clemson spinless	105.27 ab*
jo155	66.32 b-h
jo158	38.99 gh
jo161	134.09 a
jo578	67.10 c-g
jo875	42.717 gh
jo877	101.09 abc
jo880	59.167 fgh
jo881	52.87 fgh
jo882	62.16 d-h
jo883	106.26 a-d
jo885	90.09 b-f
jo887	68.80 c-g
jo891	78.87 b-f
jo892	76.0 b-f
jo893	90.02 b-f
jo894	31.5 h
jo895	73.87 b-g
jo898	60.76 e-h
jo900	94.04 b-e
jo902	76.7 b-f
jo903	78.73 b-f

* Means followed with same letter are not significantly different at $P<0.05$

Table 4. Standard statistics for AFLP primer combinations tested on the 21 okra (*Abelmoschus esculentus* L.) landraces and the control cultivar (*Clemson spinlees*)

Primer combination		Number of polymorphic loci	Total Number of loci	Polymorphism %	Size range	Polymorphic information content (PIC)	Marker Index (MI)
MseI	EcoRI						
CAT	AAG	29	93	31.2	67-625	0.23	6.76
CTG	AAG	34	83	41.0	61-645	0.26	8.79
CAA	AAG	30	78	38.5	80-457	0.31	9.25
CAC	AAG	28	98	28.6	60-537	0.27	7.61
CAA	AGG	26	92	28.3	67-540	0.23	5.95
CAC	ACC	32	109	29.4	92-617	0.26	8.46
CAT	AGG	21	72	29.2	75-425	0.29	6.09
CTG	ACC	26	97	26.8	54-595	0.25	6.84
Total/Average		227	722	31.6	54-645	0.26	7.5

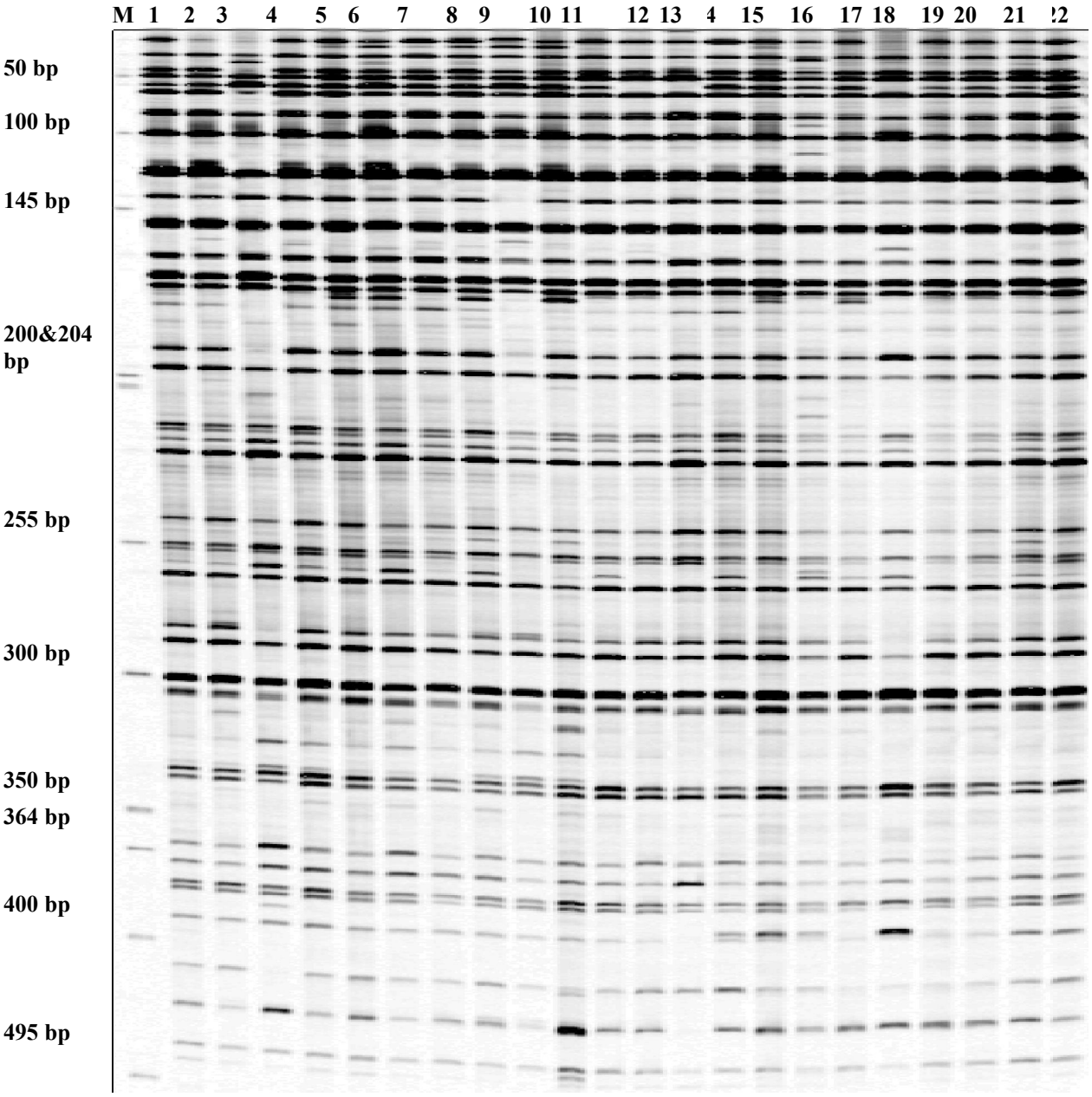

Figure 1. AFLP profile for *MseI*-CAA/*EcoRI*-AAG primer combination using the 21 Jordanian okra landraces and the control cultivar. Lanes from 1 to 22 represent landraces and the control cultivar: (1. jo155; 2. jo158; 3. jo161; 3. jo578; 4. jo875; 5. jo877; 6. jo880; 7. jo881; 8. jo882; 9. jo882; 10. jo883; 11. jo885; 12. jo887; 13. jo891; 14. jo892; 15. jo893; 16. jo894; 17. jo895; 18. jo898; 19. jo900; 20. jo902; 21. jo903; 22. *Clemson spinless*). Lane M stands for the size standard DNA marker.

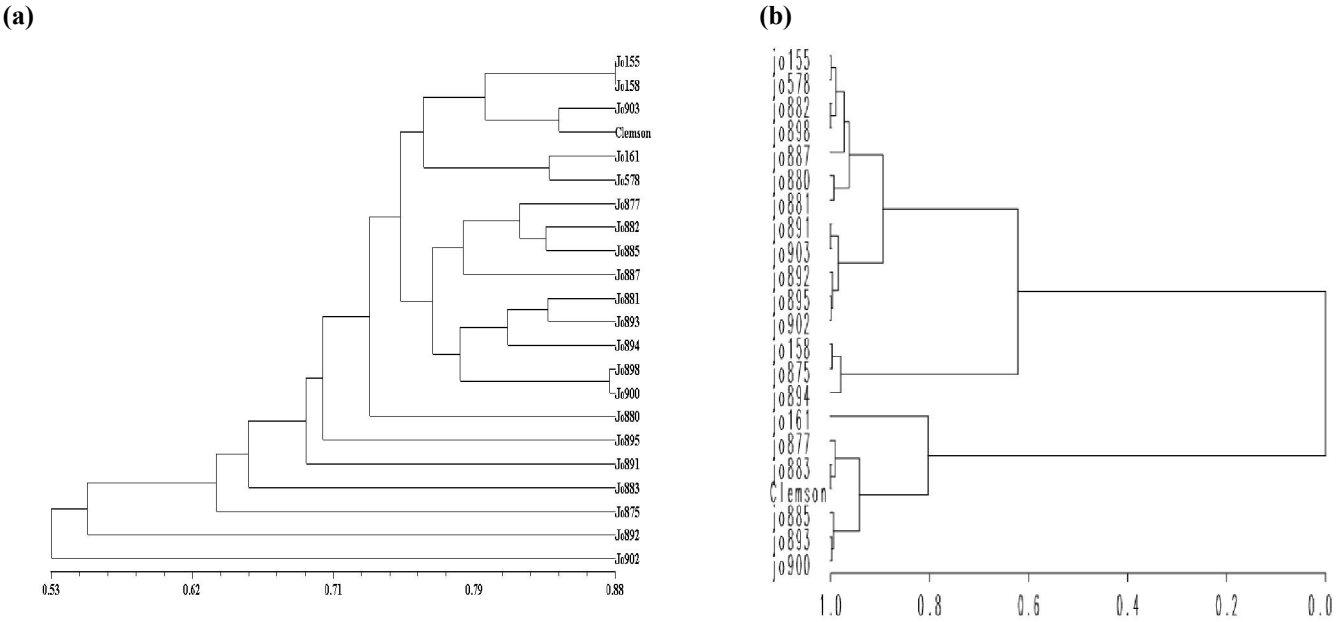

Figure 2. The Dendrogram for the 21 okra landraces and *Clemson spinless* cultivar established using (a) UPGMA method based on AFLP markers or (b) Ward's method based on morphological traits.

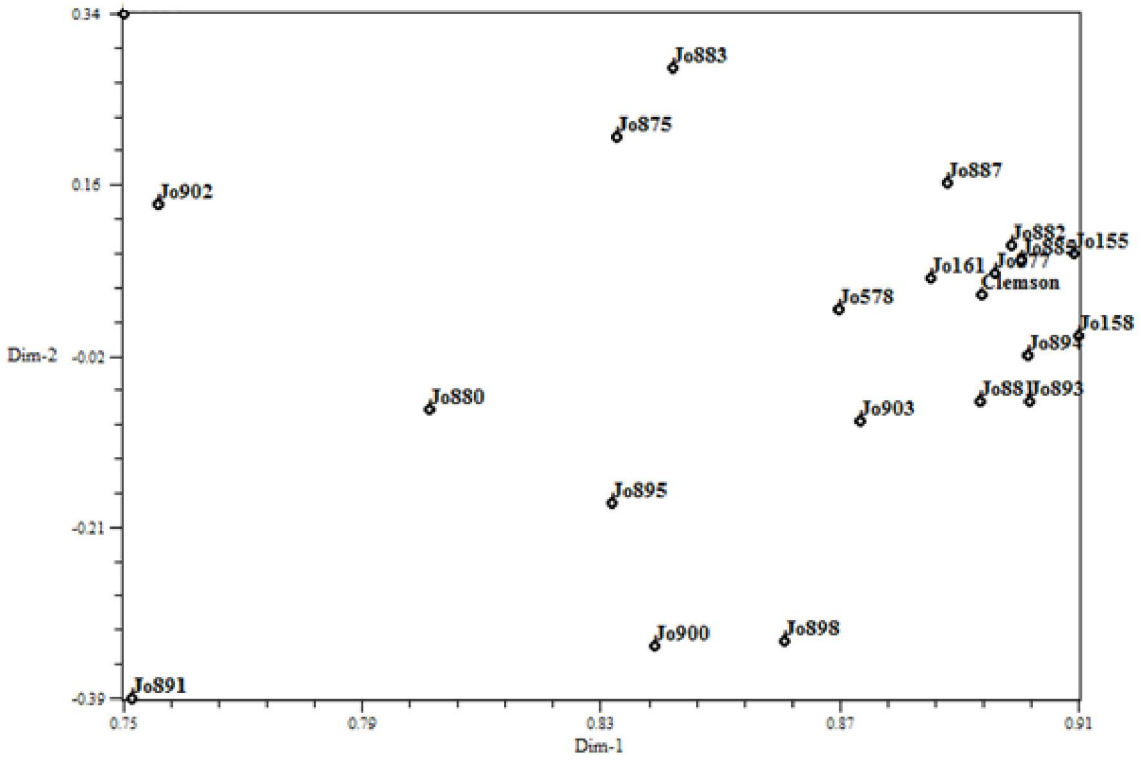

Figure 3. Principal coordinate plot of 21 okra landraces and one control cultivar (*Clemson spinless*) for the first two principal components estimated with 722 AFLP loci.

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