

## Diversity of Okra [*Abelmoschus esculentus* (L.) Moench] Genotypes in Ethiopia

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### Abstract

**Background:** Ethiopia is considered as a center of origin of okra [*Abelmoschus esculentus* (L.) Moench], but limited information is available on the genetic diversity of the crop in the country.

**Objectives:** This research was conducted to assess the genetic diversity of okra genotypes in Ethiopia.

**Materials and Methods:** Twenty-nine okra genotypes collected from Ethiopia and three varieties of the crop obtained from other countries were evaluated for 24 morpho-agronomic traits for two seasons in Ethiopia in a randomized complete block design with three replications. Besides, 19 Simple Sequences Repeats (SSRs) primers were used to assess the genetic diversity of okra genotypes.

**Results:** The genotypes showed significant differences for 24 morpho-agronomic traits. The number of days required by the genotypes to reach the first fruit harvest ranged from 55 to 84 days; the average plant height of the genotypes ranged from 106 cm to 173 cm, tender fruit weight from 14.65 g to 31.43 g, fruit yield per hectare from 0.89 t ha<sup>-1</sup> to 32.13 t ha<sup>-1</sup>, number of seeds per pod ranged from 59 to 124 and hundred seeds weight ranged from 5.3 to 7.16 g. Growing season, genotype, and year and genotype interacted to significantly influence most of the traits except internode length, leaf length, and width, number of fruits per plant and number of ridges and seeds per pod and hundred seeds weight. The four principal component axes accounted for 72.588% of the total variation with an Eigenvalue of 8.702 to 1.645. The Euclidian distances between pair of genotypes ranged from 2.36 to 12.36 as estimated from 24 morpho-agronomic traits and grouped the genotypes into nine clusters. The 18 SSRs polymorphic primers amplified 120 fragments with a size of 61 to 414 bp. Euclidean distances calculated from 120 alleles ranged from 2.2 to 7.10 and grouped the genotypes into seven distinct clusters. The distances of genotypes as estimated from SSRs and morpho-agronomic traits showed significant and positive linear relationships.

**Conclusion:** The diversity of the crop was assessed efficiently both by SSRs markers and data from morpho-agronomic traits. This implies that the genetic diversity existing among the studied okra genotypes represented varied morpho-agronomic traits, which can be used for the development high yielding new varieties for enhancing production of the vegetable crops in the country.

**Keywords:** Cluster; Euclidian distances; Morpho-agronomic traits; Simple Sequences Repeats (SSRs)

### 1. Introduction

*Abelmoschus esculentus* L. (Moench) is the only known cultivated species in the genus *Abelmoschus*. The frequently observed somatic chromosome number is  $2n = 130$ , but different authors have reported variable chromosome numbers for the species. Okra is successfully grown in hot humid areas and it is sensitive to frost and extremely low

temperatures (Tripathi *et al.*, 2011). It is a plant widely grown in tropical and subtropical countries of the world (Saifullah and Rabbani 2009; Werner *et al.*, 2016). Okra is an annual plant and requires from 90–100 days maturing. It has erect stem and varies in branching habit. The plant can attain a height of 0.5 to 4 meters. It is self-pollinating, but cross-pollination up to the extent of 4–19% has been reported (Shalaby, 1972). The plant usually bears its first



flower one to two months after sowing and flowering continues for 40–60 days depending upon the variety, the season, and soil moisture and fertility. Regular harvesting stimulates continued fruiting (Tripathi *et al.*, 2011).

Okra is a vegetable crop mainly grown for its young immature fruits. It provides carbohydrates, protein, vitamins A, B<sub>1</sub>, riboflavin,  $\beta$ -carotene, ascorbic acid, dietary fiber, minerals such as K, Na, Mg, Ca, Fe, Zn and Mn (Tripathi *et al.*, 2011, Maurya *et al.*, 2013, Priya *et al.*, 2014). The seeds are rich in the contents of tryptophan and linoleic acid (Savello *et al.*, 1980) with seeds oil content as high as 40% (Anwar *et al.*, 2011; Tripathi *et al.*, 2011). Okra is mainly cultivated for its immature pods consumed fresh or cooked, but the seeds can produce oil yield comparable to oil palm and soybean (Rao, 1985). Despite its importance as a vegetable crop and source of seed oil, the crop has been given little attention in research worldwide (Werner *et al.*, 2016). However, the total area under okra production has increased worldwide. Thus, from 2009 to 2010, the total area under okra production was 0.43 million hectares and the harvested fresh pod yield was 4.54 million tons (Varmudy 2011). However, in 2012, the okra production increased to 1,085,146 hectares and the production increased to 8,359,944 tons of fresh fruit yield (FAO, 2014). In 2018, there were about two million hectares producing almost 10 million tons of fresh okra pods worldwide (Schafleitner *et al.*, 2021).

Some scientist argued that Ethiopia is the center of origin for okra (Benchasri, 2012; Reddy *et al.*, 2012; Santos, 2012). In Ethiopia, okra is a traditional crop produced from landraces in Gambella in southwestern, Benishangul Gumuz in western, Metama and western lowland of Tigray in northwestern Ethiopia. Despite its importance and the country is considered the center of origin, the crop has not been given the required research attention in Ethiopia (MoANR, 2016). Little information is available on the diversity of the crop from research conducted very late as compared to other crops. Mihretu *et al.* (2014) reported the presence of genetic diversity among 25 okra accessions for 20 quantitative traits at Gambella and grouped into five divergent groups. It was also reported the existence of variations among 23 okra collections from Ethiopian and two exotic varieties for 25 quantitative and 10 qualitative traits evaluated at Werer in 2014 (Muluken *et al.*, 2015 and 2016). The research conducted at Melkassa in 2015 showed the variations among 50 accessions of okra for 20 and 11 qualitative and quantitative traits, respectively, and grouped into four distinct clusters (Tesfa and Yosef, 2016).

The availability of limited information demands further studies to generate information on genetic diversity of okra genotypes in Ethiopia. Moreover, the information generated was from agromorphology traits influenced by environmental factors and less effective as a tool to assess genetic diversity among germplasm than molecular markers (Chakravarthi and Naravani, 2006, Roldán-Ruiz *et al.*, 2001). Thus, molecular markers based analysis is required to provide additional information on genetic diversity, population structure and phylogenetic relationships that is independent of environmental effects (Etminan *et al.*, 2018, Khanam *et al.*, 2012, Blair *et al.*, 2009, Metais *et al.*, 2000). Information on diversity within and between crop collections is the basis for germplasm conservation and for the exploitation of useful traits in plant breeding. It is highly relevant for improving crop adaptation and overcoming yield-limiting factors (Oppong-Sekyer *et al.*, 2011) which goes beyond satisfying mere academic curiosity (Abbo *et al.*, 2010).

Genetic diversity studies based on molecular markers in okra are limited as compared to other major crops (Younis *et al.*, 2015). Kaur *et al.* (2013), Salameh (2014) and Yuan *et al.*, (2014) applied random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP) and inter-simple sequence repeat (ISSR), respectively, in okra genetic diversity analysis. Simple Sequence Repeats (SSRs) are used as the effective and cheaper options for diversity analysis of accessions of a given crop plant species (Agrama and Tuinstra, 2003), but SSRs primers for okra were developed in 2013 (Schafleitner *et al.*, 2013) and Kumar *et al.* (2017) reported diversity of okra genotypes from these markers data. Moreover, more reliable diversity information of the crop is generated both from molecular markers and from morpho-agronomic traits (Schafleitner *et al.*, 2013).

In some parts of Ethiopia such as in Gambella and Beneshangul Gumuz, okra has been cultivated for its fruit and leaf to use as a food and medicine of different diseases (Tesfa and Yosef, 2016). The landraces are under cultivation in Ethiopia and only *Bamya -Humera* has been recommended for cultivation in 2016 other than variety (SOH-701) registered by ViBHA Seed Ethiopia PLC (MoANR, 2016). Characterization of crops is the first step in any crop improvement program and the presence of significant differences among okra genotypes provides an ample scope for its improvement for horticultural traits (Oppong-Sekyer *et al.*, 2011) and germplasm conservation. Thus, this research was conducted to assess variations among okra genotypes for yield and yield

related traits and determine the extent of their genetic diversity using morpho-agronomic traits and molecular markers.

## 2. Materials and Methods

### 2.1. Experimental Materials and Testing Locations

Thirty-two okra genotypes were evaluated at Dire Dawa research site of the Haramaya University for two consecutive years (2015 and 2016). Dire Dawa is located between latitude and longitude of 9°36' N and 41°52' E

coordinates, respectively, with an altitude of 1260 meters above sea level (Hailay *et al.*, 2004). The 29 genotypes were collected by the Ethiopian Biodiversity Institute (EBI) from three geographic regions, southwestern, western and northwestern of Ethiopia at wide ranges of altitudes (490–1480 meters above sea level). In addition, two varieties from India and one variety from the United States of America were included in the study (Table 1).

Table 1. List of 29 okra collections from Ethiopian and three varieties from other countries.

| Western Ethiopia      |              |                     | Southwestern Ethiopia |          |                     |
|-----------------------|--------------|---------------------|-----------------------|----------|---------------------|
| Acc. code             | District     | Altitude (m a.s.l.) | Acc. code             | District | Altitude (m a.s.l.) |
| 92203                 | DigaLeka*    | 1200                | 240583                | Akobo    | 550                 |
| 242433                | Asossa       | 1480                | 240586                | Akobo    | 490                 |
| 242443                | Menge        | 935                 | 240587                | Akobo    | 740                 |
| 242445                | Asossa       | 725                 | 240591                | Abobo    | 630                 |
| 242449                | Asossa       | 800                 | 240585                | Akobo    | 500                 |
| 242451                | Asossa       | 1105                | 240600                | Abobo    | 490                 |
| 242444                | Menge        | 1105                | 240601                | Abobo    | 550                 |
| Northwestern Ethiopia |              |                     | 240599                | Abobo    | 480                 |
| 242203                | Mandura      | 1120                | 240602                | Abobo    | 520                 |
| 240204                | Mandura      | 1200                | 240592                | Gog      | 630                 |
| 240209                | Mandura      | 1050                | 240609                | Gambella | 730                 |
| 240201                | Mandura      | 1170                | 240615                | Gambella | 500                 |
| 240784                | Dangura      | 1180                | Other countries       |          |                     |
| 240786                | Dangura      | 1050                | SOH 704               | India    |                     |
| 245162                | Pawe Special | 1020                | SOH 701               | India    |                     |
| 245161                | Pawe Special | 1020                | Clemson spineless     | USA      |                     |
| 240207                | Dibate       | 1400                |                       |          |                     |
| 245157                | SirbAbaye    | 870                 |                       |          |                     |

Note: *Acc.* = Accession and *m a.s.l.* = Meters above sea level. The districts listed under western and northwestern Ethiopia belong to Benishangul Gumuz Region and districts listed under southwestern Ethiopia belong to Gambella Region; and \* refers to a district that belongs to Oromia Region.

### 2.2. Experimental Design and Procedures

Randomized complete block design with three replications was used and each replication consisted of 32 plots; each plot was one row consisting of 12 plants at 0.6 and 0.8 m spacing between plants and rows, respectively. Furrow irrigation was used to apply water in each plot; the experimental plots were kept free from weeds and other cultural practices such as cultivation and earthing-up were applied whenever necessary. However, no fertilizer was applied and no chemical used to control insect pests. International Plant Genetic Resources Institute (IPGRI, 1991) descriptor list for okra species was used to record data on 24 morpho-agronomic traits (Tables 3 and 4). Data for dry pod and seed traits were collected from the two border plants while data for other

traits were collected from 10 plants in the middle of the plot.

### 2.3. DNA Extraction and Amplification

This research was conducted at incotec laboratory in South Africa at the end of 2015. DNA was extracted from the bulked seed material per sample. Products of Polymerase Chain Reaction (PCR) were fluorescently labeled and separated by capillary electrophoreses on ABI 3130 automatic sequencer (Applied Biosystems Johannesburg, SA) and analysis was performed using GeneMapper 4.1. The 19 SSRs primers (Table 2) were used for okra germplasm diversity studies (Schafleitner *et al.*, 2013).

Table 2. List of SSRs primers selected for diversity analysis of 32 okra genotypes.

| Primer  | Expected fragment size (bp) | Forward primer                                      | SSR-motive  |
|---------|-----------------------------|---|---|
| AVRDC01 | 120 - 226                   | F: ATGGAGTGATTTTGTGGAG<br>R: GACCCGAACACGTTACTA     | (AAG) <sub>13</sub>   |
| AVRDC08 | 50 - 231                    | F: TGCTGTGGAAGGTTTTACT<br>R: ATGACGAAAGTGGTGA AAAAG | (AAG) <sub>8</sub>  |
| AVRDC09 | 166 - 264                   | F: ACCTTGAACACCAGGTACAG<br>R: TTGCTCTTATGAAGCAGTGA  | (AAT) <sub>12</sub>   |
| AVRDC17 | 140 - 249                   | F: ACGAGAGTGAAGTGGA ACTG<br>R: CTCCTCTTTCCCTTTTCCAT | (AGA) <sub>7</sub>  |
| AVRDC21 | 165 - 238                   | F: TCATGTCTTTCCACTCAACA<br>R: CCAAACAAAATATGCCTCTC  | (AGA) <sub>9</sub>  |
| AVRDC28 | 79 - 165                    | F: CCTCTTCATCCATCTTTTCA<br>R: GGAAGATGCTGTGAAGGTAG  | (ATT) <sub>8</sub>  |
| AVRDC39 | 104 - 144                   | F: TGAGGTGATGATGTGAGAGA<br>R: TTGTAGATGAGGTTTGAACG  | (AG) <sub>16</sub>  |
| AVRDC52 | 145 - 241                   | F: AACACATCCTCATCCTCATC<br>R: ACCGGAAGCTATTTACATGA  | (CAT) <sub>8</sub> -(TCA) <sub>9</sub>                            |
| AVRDC54 | 96 - 156                    | F: CGAAAAGGAAACTCAACAAC<br>R: TGAACCTTATTTTCCCTCGTG | (GAA) <sub>10</sub>   |
| AVRDC56 | 155 - 225                   | F: GGCAACTTCGTAATTTCCCTA<br>R: TGAGTAAAAGTGGGGTCTGT | (GAA) <sub>44</sub>   |
| AVRDC57 | 180 - 300                   | F: CGAGGAGACCATGGAAGAAG<br>R: ATGAGGAGGACGAGCAAGAA  | (GAA) <sub>9</sub> -(GAG) <sub>7</sub>                            |
| AVRDC63 | 186 - 316                   | F: GTGTTTGAAAGGGACTGTGT<br>R: CTTTCATCAAAACCATGCAG  | (TCT) <sub>12</sub>   |
| AVRDC64 | 145 - 425                   | F: AAGGAGGAGAAAGAGAAGGA<br>R: ATTTACTTGAGCAGCAGCAG  | (TCT) <sub>22</sub>   |
| AVRDC66 | 145 - 205                   | F: CACCAGAAATTTCCCTTTTG<br>R: ACTGTTGTTTGGCTTATGCT  | (TTC) <sub>12</sub> -(TTC) <sub>13</sub>                          |
| AVRDC70 | 155 - 241                   | F: GTAGCTGAACCTTTTGCTTA<br>R: CTATCATGGCGGATTTCTTTA | (TC) <sub>11</sub>  |
| AVRDC77 | 180 - 280                   | F: CTGTTTGTTCGTCGTAATCA<br>R: AAAGTTTCTTCCCTTTCCACC | (GAAATA) <sub>4</sub> -(GAAACA) <sub>7</sub>                      |
| AVRDC78 | 175 - 265                   | F: CTCGGACAATTC AAGAAAAG<br>R: CACCCAATCAAGCTATGTTA | (TAT) <sub>11</sub> -(TATTGT) <sub>4</sub> -(TATCGT) <sub>4</sub> |
| AVRDC86 | 77 - 260                    | F: ATGCAAACAAGCTAGTGGAT<br>R: ATTCTCTTCAGGGTTTCCTC  | (AGC) <sub>8</sub>  |
| AVRDC89 | 124 - 199                   | F: TTTGAGTTCCTTCGTCCACT<br>R: GTATTTGGACATGGCGTTAT  | (AGC) <sub>8</sub>  |

#### 2.4. Data Analysis

The data collected from morpho-agronomic traits were subjected to analysis of variance (ANOVA) over years. Euclidean distances (ED) of 32 okra genotypes was calculated from mean values of genotypes for 24 quantitative traits over two years after subtracting the mean value and dividing it by the standard deviation (Sneath and Sokal, 1973) as follows:

$$ED_{jk} = \sqrt{\sum_{i=1}^n (X_{ij} - X_{ik})^2}$$

Where, ED<sub>jk</sub> = distance between genotypes j and k; x<sub>ij</sub> and x<sub>ik</sub> = values of the i<sup>th</sup> phenotype traits for genotypes j and k, respectively; and n = number of

phenotype traits used to calculate the distance. The distance matrix from morpho-agronomic traits was used to construct a dendrogram based on the Unweighted Pair-group Method with Arithmetic Means (UPGMA).

Polymorphisms were treated as binary data (presence or absence). The binary data were used to obtain a dissimilarity matrix using the Jaccard index. The program GGT 2.0 (Van Berioo, 2008) was used to calculate the Euclidian distances between genotypes. A dendrogram was then generated from a dissimilarity matrix based on the UPGMA cluster analysis method by employing the software DARwin 5.0 (Perrier and Jacquemoud-Collet,

2006). GENALEX version 6.5 (Peakall and Smouse, 2012) software was used to calculate major allele frequency, allele number and availability, gene diversity ( $H_e$ ), heterozygosity ( $H_o$ ), polymorphism information content (PIC) and inbreeding coefficient (F) from the data generated from 18 SSRs polymorphic primers. In addition, regression analysis was conducted to understand the relationships between genetic distances (Euclidian distances) estimated from data of morpho-agronomic traits and SSRs markers.

### 3. Results and Discussion

#### 3.1. Analysis of Variance and Mean Performance of Genotypes

The genotypes exhibited significant differences for 24 morpho-agronomic traits (Table 3). The growing year and interaction of genotype x year also had significant influence on all traits except internode length, leaf length and width, number of seeds per pod and hundred seeds weight. Therefore, the observed significant difference among the evaluated okra genotypes is a good opportunity to develop varieties for varied traits. The genotypes had differential performances across growing seasons due to the significant influence of growing year

and interaction of genotype x year for most of the traits. This suggested the importance of evaluation of genotypes over seasons before recommending for further advanced and multilocation research. Ariyo and Vaughan (2000) and Jindal *et al.* (2008) have reported the significant influence of genotype and genotype by environment interaction on okra fruit yield and other traits.

The genotypes showed a wide range of differences in 24 morpho-agronomic traits. Moreover, extreme variation among the genotypes observed for tender fruits yield in the range between 0.89 to 32.13 t ha<sup>-1</sup> (Table 4). This research result indicated selection of okra genotypes for high fruit yield is possible to develop commercial varieties. The fruit yield of okra in most of the developing countries is low (1.77 t ha<sup>-1</sup>) as compared to the yield of other agriculturally developed countries (as high as 30 t ha<sup>-1</sup>) (Whitehead and Singh, 2000). *Bamya-Humera* variety produced 18.47 and up to 16 t ha<sup>-1</sup> green pod at research and farmers field, respectively (MoANR, 2016). Other authors also reported the presence of a wide range of variation among okra genotypes in Ethiopia (Mihretu *et al.*, 2014; Muluken *et al.*, 2015, 2016; Tesfa and Yosef, 2016).

Table 3. Mean squares for combined analysis of variance over two years (2015 and 2016) for 24 quantitative traits of 32 okra genotypes at Dire Dawa, Ethiopia.

| Trait                        | Replication<br>(DF = 2) | Genotype (G)<br>(DF = 31) | Year (Y)<br>(DF = 1) | G x Y<br>(DF = 31) | Error<br>(DF = 126) | CV<br>(%) |
|------------------------------|-------------------------|---------------------------|----------------------|--------------------|---------------------|-----------|
| Days to first flower bud set | 25.52                   | 256.87**                  | 833.56**             | 74.12**            | 21.03               | 11.43     |
| Days to first flower set     | 35.78                   | 326.25**                  | 455.45**             | 78.21**            | 24.18               | 8.72      |
| Days to first fruit set      | 39.22                   | 339.69**                  | 140.42*              | 80.45**            | 25.18               | 8.51      |
| Days to first fruit harvest  | 45.57                   | 369.08**                  | 194.01*              | 86.07**            | 26.98               | 8.01      |
| Number of harvest            | 0.188                   | 14.469**                  | 115.63**             | 6.576**            | 1.352               | 14.3      |
| Duration of harvest          | 10.2                    | 590.11**                  | 15226.7**            | 251.12**           | 51.52               | 13.6      |
| Plant height (cm)            | 0.02112                 | 0.19596**                 | 0.86717**            | 0.02434            | 0.02911             | 12.4      |
| Number of primary branches   | 1.0845                  | 13.9589**                 | 25.6913**            | 2.4946**           | 0.4549              | 12.7      |
| Stem diameter (cm)           | 0.0406                  | 0.321**                   | 5.9162**             | 0.2795**           | 0.1009              | 16,03     |
| Internode length (cm)        | 1.918                   | 23.607**                  | 5.957                | 0.014              | 1.183               | 15.11     |
| Leaf length (cm)             | 12.773                  | 38.275**                  | 19.533               | 0.023              | 3.533               | 14.42     |
| Leaf width (cm)              | 1191.99                 | 61.13**                   | 18.78                | 0.04               | 12.36               | 27.41     |
| Number of fruit per plant    | 37.95                   | 212.306**                 | 12697.743**          | 171.602**          | 8.928               | 13.8      |
| Number of fruit per harvest  | 1.9926                  | 1.835**                   | 121.9881**           | 1.359**            | 0.3649              | 23.22     |
| Tender fruit weight (g)      | 24.917                  | 113.788**                 | 11.747               | 47.442**           | 8.637               | 12.81     |
| Tender fruit length (cm)     | 5.466                   | 44.963**                  | 26.095**             | 7.782**            | 1.455               | 11.43     |
| Tender fruit width (cm)      | 0.08203                 | 2.97332**                 | 2.10865**            | 0.40316**          | 0.06507             | 10.03     |
| Number of ridges on fruit    | 2.8581                  | 4.5032**                  | 0.0182               | 0.0088             | 0.7942              | 12.42     |
| Fruit yield per harvest (g)  | 4.69                    | 1942.56**                 | 78921.54**           | 1605.78**          | 31.7                | 9.51      |
| Fruit yield per hectare (t)  | 57.446                  | 182.024**                 | 298.655**            | 19.322**           | 5.451               | 14.91     |
| Number of dry pod per plant  | 237.13                  | 130.17**                  | 10862.5*             | 131.87**           | 46.11               | 18.92     |
| Dry pod weight (g)           | 3.603                   | 61.547*                   | 16.251**             | 15.805**           | 3.367               | 14.21     |
| Number of seeds per pod      | 204.3                   | 1186**                    | 979.7                | 194.7              | 358.3               | 19.32     |
| Hundred seeds weight (g)     | 0.5904                  | 1.552**                   | 0.5682               | 0.2903             | 0.6968              | 13.34     |

Note: \* and \*\* refer to statistical significance at  $P < 0.05$  and  $P < 0.01$ , respectively. DF = Degrees of freedom. CV = Coefficient of variation (%).

The genotypes showed wide variations for days to first flower set in the range between 47 and 74 days after sowing and duration of harvest ranged from 26 to 68 days (Table 4). The observed wide variations among the genotypes enable breeders to develop high-yielding early maturing to late maturing ones with long durations of harvest. One of the objectives of okra improvement is to breed for early maturing and late senescing varieties to increase the duration of photo-assimilation and productivity of the crop. In other countries, okra varieties open the first flower in the range between 41 and 48 days after sowing. Once the plant starts flowering, the crop continues flowering for 40 to 60 days (Sulikeri and Swamy, 1972; Tripathi *et al.*, 2011). Thus, the genotypes evaluated in this study flowered relatively late that extended from 47 to 74 days after sowing with wide variations from short to extended durations of flowering. The variations in duration of flowering might be due to the inherent genetic factors of the genotypes and the influence of the growing season. The first flower and continued flowering of plants depends on variety, the season, soil moisture and fertility as well as the regular harvesting of fruits on plants (Tripathi *et al.*, 2011).

The genotypes had tender fruit length and width that ranged from 6.93 cm to 20.49 cm and 1.77 cm to 5.89 cm,

respectively. The tender and immature fruits that grow rapidly into long 10 cm to 30 cm and narrow from 1 cm to 4 cm are harvested for consumption as vegetable (Tripathi *et al.*, 2011). The genotypes also had number of seeds per pod that ranged from 59 to 124 that can enable developing varieties that combines desirable traits of long and wide fruits with many number of seeds. The tender fruits along with the seeds are processed and consumed as stew and salad, soup, sliced, boiled and fried vegetables (Akanbi *et al.*, 2010). The seeds protein is rich in tryptophan and it contains adequate amounts of sulfur-containing amino acid, which is a rare combination that makes okra seeds exceptionally useful in reducing human malnutrition. Edible oil rich in unsaturated fatty acids (60% to 70%) can be produced from okra seed (Savello *et al.*, 1980; Rao, 1985), and seed flour of okra can be used to fortify cereal flour (Adelakun *et al.*, 2009). The level of genetic variation present in crop germplasm is proportional to its improvement (Aladele, 2009). Thus, the observed wide variation among okra genotypes for all morpho-agronomic traits is a good indicator for the improvement of okra in Ethiopia through collection, characterization, and evaluation.

Table 4. Range and mean values of 32 okra genotypes for 24 quantitative traits evaluated in 2015 and 2016 at Dire Dawa, Ethiopia.

| Trait                        | Minimum | Maximum | Mean  | LSD (5%) |
|------------------------------|---------|---------|-------|----------|
| Days to first flower bud set | 32      | 55      | 40    | 7.4      |
| Days to first flower set     | 47      | 74      | 57    | 7.9      |
| Days to first fruit set      | 50      | 77      | 59    | 8.1      |
| Days to first fruit harvest  | 55      | 84      | 65    | 8.39     |
| Number of harvest            | 4       | 11      | 8     | 1.9      |
| Duration of harvest          | 26      | 68      | 53    | 11.6     |
| Plant height (cm)            | 106     | 173     | 137   | 28       |
| Primary branches             | 3       | 10      | 5     | 1.1      |
| Stem diameter (cm)           | 1.33    | 2.67    | 2     | 0.51     |
| Internode length (cm)        | 4.72    | 11.69   | 7.22  | 1.76     |
| Leaf length (cm)             | 7.18    | 17.53   | 13.08 | 3.04     |
| Leaf width (cm)              | 4.09    | 18.42   | 12.83 | 5.68     |
| Number of fruit per plant    | 2       | 30      | 22    | 4.8      |
| Number of fruit per harvest  | 1       | 4       | 3     | 1        |
| Tender fruit weight (g)      | 14.65   | 31.43   | 23.04 | 4.75     |
| Tender fruit length (cm)     | 6.93    | 20.49   | 10.56 | 1.95     |
| Tender fruit width (cm)      | 1.77    | 5.89    | 2.54  | 0.41     |
| Number of ridges on fruit    | 4       | 8       | 7     | 1.4      |
| Fruit yield per harvest (g)  | 10.86   | 89.23   | 59.04 | 9.1      |
| Fruit yield per hectare (t)  | 0.89    | 32.13   | 15.67 | 3.77     |
| Number of dry pod per plant  | 2       | 25      | 17    | 11       |
| Dry pod weight (g)           | 7.09    | 19.73   | 12.88 | 2.97     |

|                          |      |      |      |      |
|--------------------------|------|------|------|------|
| Number of seeds per pod  | 59   | 124  | 98   | 30.6 |
| Hundred seeds weight (g) | 5.23 | 7.16 | 6.26 | 1.35 |

Note: *LSD* = *Least significant difference* at  $P < 0.05$ .

### 3.2. Principal Component Analysis

The four principal component axes accounted 72.588% of the total variation with an Eigenvalue of 8.702 to 1.645. The first three PCAs had a cumulative contribution of 65.734% in which PCA1, PCA2 and PCA3 contributed 36.257, 18.735 and 10.742% to the total variability, respectively. However, PCA4 contributed 6.854% to the observed variability among the 32 okra genotypes. Number of harvests, days to first flower set, days to first fruit set, number of fruit per plant, days to first flower bud set, duration of harvest, number of dry pods per plant, days to first fruit harvest and number of primary branches had largest contribution to the PCA1. Dry pod weight, tender fruit weight and number of seeds per pod in PCA2, and tender fruit length in PCA3 had the largest absolute values 0.702 to 0.945 (Table 5). This indicated these traits had the largest contribution to the clustering of genotypes into different groups. The traits with the largest absolute values closer to unity in each principal component axis influence the clustering more than PCAs with lower absolute values closer to zero (Chahal and Gosal, 2002). In addition, fruit yield per harvest, fruit yield per hectare, number of ridges on fruit in PCA2, internode length and leaf width in PCA4 and number of fruit per

harvest, leaf length, plant height and tender fruit width in PCA1 had the largest squared cosine. Thus, these traits had also a larger cumulative effect to the differentiation of the genotypes into different clusters than other traits.

Average fruit weight, leaf length, number of seeds per fruit, stem diameter, leaf width, number of internodes, plant height, fruit diameter and tender fruit yield had the largest contribution to the total variation of okra genotypes as reported by Kumari *et al.* (2019). Mudhalvan and Senthilkumar (2018) also reported that the number of fruits per plant followed by the number of branches per plant, fruit girth, days to fruit maturity and days to first flowering contributed the maximum towards the genetic divergence. Nwangburuka *et al.* (2012) identified days to flowering, branches per plant, fruit diameter and seeds per pod had relatively higher contribution in the principal axes. Plant height and number of pods per plant had relatively high contributions in the principal axes (Ahiakpa *et al.*, 2013). Principal component analysis reflects the importance of the largest contributor to the total variation at each axis for differentiation of genotypes (Sharma, 1998).

Table 5. Factor loadings, contribution of traits and Eigen values of four principal component axes in 32 okra genotypes evaluated at Dire Dawa in 2015 and 2016.

| Trait   | PCA1          | PCA2         | PCA3         | PCA4         |
|---|---------------|--------------|--------------|--------------|
| Days to first flower bud set                  | <b>-0.893</b> | 0.181        | -0.315       | -0.168       |
| Days to first flower set                      | <b>-0.914</b> | 0.170        | -0.266       | -0.164       |
| Days to first fruit set                       | <b>-0.909</b> | 0.175        | -0.281       | -0.168       |
| Days to first fruit harvest                   | <b>-0.833</b> | 0.223        | -0.258       | -0.161       |
| Number of dry pod per plant                   | <b>0.848</b>  | 0.115        | -0.408       | -0.095       |
| Dry pod weight (g)                            | 0.046         | <b>0.842</b> | 0.304        | -0.177       |
| Duration of harvest                           | <b>0.887</b>  | -0.008       | 0.387        | 0.015        |
| Tender fruit width (cm)                       | <b>-0.425</b> | -0.033       | -0.097       | -0.196       |
| Tender fruit length (cm)                      | 0.041         | 0.143        | <b>0.787</b> | -0.090       |
| Fruit yield per harvest (g)                   | 0.538         | <b>0.674</b> | -0.349       | -0.168       |
| Fruit yield per hectare (t ha <sup>-1</sup> ) | 0.260         | <b>0.627</b> | -0.416       | 0.192        |
| Hundred seeds weight (g)                      | -0.175        | 0.503        | 0.295        | 0.214        |
| Internode length (cm)                         | 0.077         | 0.549        | -0.203       | <b>0.606</b> |
| Leaf length (cm)                              | <b>-0.518</b> | 0.288        | 0.261        | 0.094        |
| Leaf width (cm)                               | -0.232        | 0.550        | -0.218       | <b>0.560</b> |
| Number of ridges on fruit                     | 0.095         | <b>0.607</b> | 0.307        | -0.259       |
| Number of fruit per harvest                   | <b>0.666</b>  | 0.275        | -0.564       | -0.236       |
| Number of fruit per plant                     | <b>0.902</b>  | 0.130        | -0.316       | -0.123       |
| Number of harvest                             | <b>0.945</b>  | -0.006       | 0.161        | -0.046       |
| Number of primary branches                    | <b>-0.749</b> | -0.138       | 0.111        | 0.112        |
| Number of seeds per pod                       | -0.039        | <b>0.702</b> | 0.231        | -0.386       |
| Plant height (cm)                             | <b>-0.497</b> | 0.434        | 0.193        | 0.311        |
| Tender fruit weight (g)                       | 0.158         | <b>0.809</b> | 0.213        | -0.077       |
| Stem diameter (cm)                            | -0.387        | 0.081        | -0.005       | -0.494       |
| Eigenvalue                                    | 8.702         | 4.496        | 2.578        | 1.645        |
| Contribution to variability (%)               | 36.257        | 18.735       | 10.742       | 6.854        |
| Cumulative contribution (%)                   | 36.257        | 54.992       | 65.734       | 72.588       |

Note: PCA = Principal component axis. Values in bold correspond for each variable to the factor for which the squared cosine is the largest.

### 3.3. Euclidian Distances and Clustering of Genotypes based on Phenotype Traits

A wide range of Euclidian distances that ranged from 2.36 (between 240209 and 240591) to 12.36 (between 240786 and 240600) was computed from 24 morpho-agronomic traits. The overall mean Euclidian distance of genotypes was 6.13 with standard deviation and coefficient of variation of 1.86 and 30.27%, respectively. The higher Euclidian distances were estimated among okra genotypes collected from Ethiopia than Euclidian distances estimated among commercial varieties introduced from other countries (Table 6). Dendrogram generated from Euclidian distances matrix based on UPGMA clustering method identified nine distinct clusters with 48.92% within-class and 51.08% between class variance decomposition for the optimal classification. Five solitary clusters were identified, and Cluster I and V consisted of each two genotypes. Cluster IV and VI consisted of 8 (25%) and 15 (46.88%), respectively, and all the three introduced commercial varieties were grouped under cluster IV (Figure 1).

The observed wide ranges of genetic distances among okra genotypes and clustering of genotypes into different groups showed the abundance of genetic diversity of the crop in Ethiopia. This is a good opportunity for breeders to develop varieties either through selection and/or hybridization. Characterization and assessing the diversity of crop species is the first and crucial step since the success of any crop improvement programs is a function of the level of the available genetic diversity in the crop (Oppong-Sekyere *et al.*, 2011). The present study also encourages further collection of okra germplasm for germplasm conservation in the country. Other authors (Mihretu *et al.*, 2014; Muluken *et al.*, 2015, 2016; Tesfa and Yosef, 2016) reported similar results. This may support the scientists who argued the country is the center of origin for okra (Benchasri, 2012; Reddy *et al.*, 2012). Certain areas that exhibit high level of genetic variability for crops are considered as center of genetic diversity (Falconer and Mackay, 1996; Eivazi *et al.*, 2007) and genetic diversity is associated with center of origin (Christine *et al.*, 2009).



Table 6. Euclidian distances of 32 okra genotypes estimated from 24 morpho-agronomic traits (above diagonal) 18 polymorphic SSRs primers (below diagonal).

| No | Genotype | 1    | 2    | 3    | 4    | 5    | 6    | 7    | 8    | 9    | 10   | 11   | 12   | 13   | 14    | 15   | 16   |
|----|----------|------|------|------|------|------|------|------|------|------|------|------|------|------|-------|------|------|
| 1  | 92203    |      | 6.98 | 5.70 | 3.45 | 4.72 | 4.55 | 7.46 | 4.37 | 4.74 | 4.68 | 3.13 | 3.79 | 6.79 | 7.40  | 3.84 | 5.59 |
| 2  | 240201   | 5.20 |      | 6.56 | 5.40 | 5.90 | 4.71 | 4.02 | 8.74 | 5.21 | 5.74 | 6.71 | 6.07 | 4.74 | 8.35  | 5.60 | 5.85 |
| 3  | 240204   | 4.20 | 4.20 |      | 5.65 | 7.51 | 5.04 | 7.69 | 7.36 | 4.70 | 7.62 | 5.58 | 5.68 | 6.30 | 10.63 | 6.84 | 4.08 |
| 4  | 240207   | 4.60 | 4.60 | 3.60 |      | 4.76 | 4.00 | 6.23 | 6.03 | 4.51 | 4.40 | 4.94 | 5.17 | 6.46 | 7.48  | 3.77 | 5.40 |
| 5  | 240209   | 4.20 | 5.50 | 3.00 | 4.70 |      | 5.26 | 6.23 | 5.29 | 5.36 | 2.36 | 4.79 | 4.62 | 7.01 | 5.56  | 3.84 | 6.63 |
| 6  | 240583   | 4.10 | 4.70 | 3.40 | 4.60 | 3.90 |      | 5.24 | 7.20 | 3.05 | 5.30 | 5.41 | 4.71 | 4.73 | 8.64  | 5.71 | 5.40 |
| 7  | 240585   | 4.50 | 4.80 | 5.00 | 5.40 | 5.10 | 3.90 |      | 9.70 | 5.98 | 5.64 | 6.91 | 7.22 | 4.67 | 8.72  | 6.63 | 5.96 |
| 8  | 240586   | 5.30 | 6.10 | 4.40 | 4.80 | 4.20 | 5.10 | 5.70 |      | 6.58 | 6.01 | 5.02 | 4.98 | 9.53 | 8.58  | 5.54 | 7.37 |
| 9  | 240587   | 4.40 | 5.00 | 3.10 | 4.60 | 3.70 | 2.90 | 4.10 | 4.10 |      | 5.47 | 5.06 | 3.38 | 4.89 | 8.92  | 5.82 | 5.08 |
| 10 | 240591   | 4.30 | 4.90 | 4.80 | 5.50 | 4.70 | 5.20 | 4.20 | 4.20 | 4.90 |      | 4.58 | 4.80 | 6.92 | 5.59  | 3.44 | 5.96 |
| 11 | 240592   | 4.80 | 4.80 | 5.40 | 5.90 | 4.60 | 4.80 | 4.20 | 5.10 | 4.80 | 3.60 |      | 3.62 | 6.10 | 7.32  | 3.86 | 4.99 |
| 12 | 240599   | 4.80 | 4.80 | 3.80 | 4.40 | 3.40 | 4.60 | 5.10 | 3.40 | 3.50 | 4.10 | 4.40 |      | 5.87 | 7.57  | 4.40 | 5.83 |
| 13 | 240600   | 3.30 | 5.00 | 4.50 | 4.90 | 4.80 | 4.80 | 4.60 | 5.90 | 5.20 | 4.60 | 4.90 | 5.30 |      | 8.67  | 6.82 | 6.49 |
| 14 | 240601   | 5.20 | 5.40 | 5.60 | 5.90 | 5.50 | 5.90 | 4.70 | 4.50 | 5.40 | 3.10 | 4.70 | 4.70 | 4.80 |       | 5.91 | 9.45 |
| 15 | 240602   | 4.60 | 5.40 | 4.90 | 5.80 | 4.60 | 4.90 | 5.00 | 4.20 | 4.90 | 3.10 | 3.60 | 4.10 | 5.00 | 4.80  |      | 6.17 |
| 16 | 240609   | 4.80 | 3.70 | 4.70 | 4.70 | 4.30 | 4.30 | 5.40 | 5.30 | 5.00 | 4.70 | 3.40 | 3.70 | 5.10 | 5.20  | 4.40 |      |
| 17 | 240615   | 3.00 | 5.10 | 4.10 | 4.70 | 4.30 | 3.80 | 4.80 | 5.30 | 4.30 | 4.10 | 4.20 | 4.60 | 3.50 | 5.40  | 4.70 | 4.20 |
| 18 | 240784   | 5.50 | 7.10 | 5.50 | 5.40 | 4.70 | 5.40 | 5.10 | 4.40 | 5.10 | 4.80 | 5.20 | 4.50 | 5.60 | 6.20  | 4.30 | 5.90 |
| 19 | 240786   | 5.10 | 5.90 | 5.20 | 5.00 | 4.80 | 4.80 | 6.00 | 5.00 | 5.40 | 5.30 | 6.10 | 4.60 | 5.80 | 6.10  | 5.50 | 4.90 |
| 20 | 242203   | 4.30 | 3.80 | 3.40 | 3.90 | 4.50 | 4.90 | 5.20 | 5.80 | 4.80 | 4.80 | 6.00 | 4.30 | 4.80 | 5.70  | 5.20 | 4.90 |
| 21 | 242433   | 4.40 | 4.20 | 3.40 | 4.20 | 3.50 | 3.80 | 5.20 | 4.60 | 3.90 | 4.60 | 5.00 | 2.80 | 4.40 | 5.30  | 5.00 | 3.60 |
| 22 | 242443   | 4.40 | 4.70 | 4.00 | 5.10 | 3.50 | 4.00 | 4.60 | 3.80 | 3.40 | 4.00 | 2.80 | 3.00 | 5.20 | 4.60  | 3.40 | 3.30 |
| 23 | 242444   | 5.10 | 4.80 | 6.40 | 5.90 | 6.00 | 5.60 | 3.80 | 6.20 | 6.10 | 4.20 | 3.50 | 5.00 | 5.30 | 5.10  | 4.40 | 4.20 |
| 24 | 242445   | 5.60 | 4.40 | 5.20 | 5.70 | 4.80 | 5.10 | 5.10 | 5.40 | 5.10 | 4.00 | 2.90 | 4.10 | 5.00 | 4.90  | 4.20 | 3.60 |
| 25 | 242449   | 4.90 | 4.00 | 5.10 | 5.10 | 5.00 | 4.60 | 4.90 | 5.10 | 4.50 | 4.20 | 3.20 | 3.20 | 5.00 | 4.90  | 4.40 | 2.60 |
| 26 | 242451   | 4.70 | 4.40 | 5.20 | 5.70 | 5.10 | 5.40 | 4.60 | 4.80 | 5.30 | 2.20 | 3.20 | 4.50 | 4.60 | 3.50  | 3.00 | 4.20 |
| 27 | 245157   | 3.40 | 4.50 | 3.30 | 3.50 | 4.40 | 2.80 | 4.10 | 5.40 | 3.30 | 4.80 | 5.10 | 4.70 | 4.00 | 5.30  | 5.50 | 4.60 |
| 28 | 245161   | 4.20 | 4.80 | 3.50 | 4.20 | 3.70 | 4.20 | 4.60 | 4.80 | 4.70 | 4.70 | 5.70 | 3.80 | 4.60 | 5.10  | 4.90 | 4.70 |
| 29 | 245162   | 5.40 | 3.40 | 5.20 | 5.30 | 5.30 | 5.10 | 4.70 | 6.20 | 6.10 | 4.60 | 4.80 | 4.80 | 5.00 | 5.20  | 5.00 | 4.00 |
| 30 | Clemson  | 5.10 | 5.10 | 5.60 | 5.10 | 5.60 | 5.90 | 5.20 | 6.10 | 5.90 | 5.00 | 4.80 | 4.40 | 4.70 | 6.00  | 4.30 | 4.80 |
| 31 | SOH701   | 4.00 | 3.50 | 4.50 | 4.30 | 5.00 | 5.20 | 4.90 | 6.00 | 5.50 | 4.40 | 5.00 | 4.50 | 3.50 | 4.20  | 5.70 | 3.90 |
| 32 | SOH714   | 3.30 | 4.60 | 4.50 | 4.40 | 4.40 | 4.60 | 3.70 | 5.30 | 4.80 | 3.50 | 3.60 | 4.70 | 3.40 | 4.40  | 4.70 | 4.20 |

Table 6. Continued.

| No | Genotype | 17   | 18   | 19    | 20   | 21   | 22    | 23    | 24    | 25    | 26   | 27   | 28   | 29   | 30    | 31    | 32    |
|----|----------|------|------|-------|------|------|-------|-------|-------|-------|------|------|------|------|-------|-------|-------|
| 1  | 92203    | 4.69 | 4.25 | 9.83  | 2.65 | 6.58 | 4.79  | 6.67  | 8.06  | 7.20  | 4.24 | 4.38 | 5.53 | 4.70 | 7.11  | 6.47  | 7.80  |
| 2  | 240201   | 5.81 | 4.64 | 9.94  | 5.72 | 5.80 | 4.58  | 4.86  | 6.43  | 8.35  | 8.99 | 3.52 | 3.95 | 6.13 | 5.42  | 5.12  | 5.59  |
| 3  | 240204   | 4.95 | 6.69 | 12.27 | 5.95 | 8.30 | 4.78  | 6.42  | 10.08 | 10.99 | 8.35 | 6.21 | 7.31 | 5.43 | 4.91  | 5.52  | 7.69  |
| 4  | 240207   | 5.03 | 3.31 | 9.42  | 4.07 | 6.44 | 4.46  | 5.81  | 7.87  | 7.50  | 6.13 | 3.74 | 5.02 | 5.17 | 6.85  | 5.80  | 7.25  |
| 5  | 240209   | 5.49 | 3.60 | 7.36  | 3.64 | 4.58 | 5.87  | 6.60  | 6.42  | 5.70  | 5.88 | 3.36 | 4.03 | 4.79 | 6.94  | 7.33  | 7.19  |
| 6  | 240583   | 5.83 | 4.69 | 10.94 | 4.50 | 6.88 | 4.97  | 6.00  | 7.62  | 9.22  | 7.95 | 3.92 | 5.27 | 5.83 | 5.79  | 4.71  | 5.75  |
| 7  | 240585   | 7.16 | 5.83 | 10.33 | 6.86 | 7.43 | 5.36  | 4.20  | 6.33  | 8.84  | 8.92 | 4.37 | 5.02 | 6.81 | 5.19  | 4.44  | 3.98  |
| 8  | 240586   | 5.74 | 5.55 | 10.09 | 4.48 | 7.48 | 6.85  | 8.94  | 9.35  | 8.23  | 5.89 | 6.48 | 7.10 | 4.84 | 8.71  | 9.03  | 9.77  |
| 9  | 240587   | 5.51 | 4.49 | 11.25 | 4.18 | 7.18 | 4.16  | 6.33  | 7.60  | 9.44  | 7.59 | 4.44 | 5.88 | 4.84 | 5.13  | 4.35  | 5.41  |
| 10 | 240591   | 5.28 | 3.53 | 6.89  | 3.94 | 5.28 | 5.37  | 5.97  | 6.07  | 5.42  | 5.57 | 3.06 | 4.24 | 4.93 | 6.93  | 6.56  | 6.88  |
| 11 | 240592   | 4.34 | 4.85 | 9.48  | 2.85 | 5.87 | 4.00  | 5.32  | 6.94  | 6.79  | 4.15 | 4.20 | 4.99 | 4.01 | 5.62  | 5.54  | 7.11  |
| 12 | 240599   | 4.37 | 4.30 | 10.31 | 2.45 | 6.02 | 4.14  | 6.50  | 7.30  | 7.62  | 5.73 | 4.18 | 5.54 | 3.56 | 5.97  | 5.81  | 6.98  |
| 13 | 240600   | 7.13 | 6.67 | 12.36 | 5.89 | 6.93 | 4.58  | 4.99  | 6.24  | 9.19  | 8.55 | 4.96 | 5.34 | 6.87 | 4.60  | 3.48  | 4.58  |
| 14 | 240601   | 8.00 | 6.72 | 7.95  | 6.52 | 5.53 | 8.26  | 8.71  | 6.74  | 4.55  | 6.64 | 6.10 | 5.69 | 8.22 | 9.69  | 9.71  | 9.91  |
| 15 | 240602   | 3.71 | 3.00 | 7.89  | 3.37 | 4.37 | 4.38  | 5.14  | 7.24  | 4.74  | 4.31 | 2.88 | 4.05 | 4.02 | 6.96  | 6.70  | 8.12  |
| 16 | 240609   | 4.38 | 5.72 | 9.99  | 5.76 | 8.06 | 4.17  | 5.53  | 8.63  | 9.74  | 7.37 | 5.26 | 6.32 | 5.14 | 4.50  | 4.98  | 6.47  |
| 17 | 240615   |      | 4.17 | 9.18  | 4.62 | 5.84 | 4.09  | 5.54  | 9.02  | 7.72  | 5.60 | 4.19 | 5.47 | 3.19 | 5.88  | 6.62  | 8.57  |
| 18 | 240784   | 5.40 |      | 8.26  | 3.69 | 5.10 | 4.44  | 5.81  | 7.10  | 6.41  | 5.87 | 2.57 | 3.87 | 4.38 | 6.75  | 6.33  | 7.18  |
| 19 | 240786   | 5.00 | 5.50 |       | 9.19 | 8.24 | 10.55 | 10.22 | 10.13 | 7.00  | 9.04 | 8.40 | 8.36 | 9.75 | 11.30 | 12.11 | 11.99 |
| 20 | 242203   | 5.20 | 6.10 | 4.90  |      | 5.12 | 4.21  | 6.19  | 6.31  | 6.25  | 5.00 | 3.45 | 4.34 | 4.19 | 6.39  | 6.04  | 6.85  |
| 21 | 242433   | 3.80 | 5.10 | 4.00  | 3.90 |      | 6.67  | 6.66  | 6.60  | 5.14  | 6.81 | 4.30 | 3.56 | 6.37 | 7.61  | 8.15  | 9.02  |
| 22 | 242443   | 4.50 | 4.90 | 5.40  | 5.20 | 4.20 |       | 3.99  | 7.04  | 7.89  | 6.22 | 3.76 | 4.87 | 3.92 | 4.33  | 3.75  | 5.76  |
| 23 | 242444   | 5.50 | 5.50 | 6.20  | 5.70 | 5.60 | 4.60  |       | 7.51  | 8.00  | 7.34 | 4.36 | 5.28 | 5.24 | 4.17  | 4.15  | 6.15  |
| 24 | 242445   | 4.60 | 5.20 | 5.60  | 5.60 | 4.30 | 3.50  | 4.60  |       | 6.96  | 8.82 | 5.87 | 4.93 | 8.45 | 8.02  | 6.99  | 6.00  |
| 25 | 242449   | 4.10 | 5.40 | 4.90  | 5.30 | 3.50 | 3.20  | 3.90  | 2.50  |       | 5.59 | 6.00 | 5.59 | 7.84 | 10.01 | 9.88  | 10.26 |
| 26 | 242451   | 4.80 | 5.50 | 5.70  | 5.00 | 5.00 | 3.80  | 4.00  | 3.40  | 3.90  |      | 6.00 | 6.61 | 5.63 | 8.38  | 8.40  | 9.77  |
| 27 | 245157   | 3.10 | 5.90 | 5.20  | 4.30 | 3.90 | 4.70  | 5.80  | 5.10  | 4.60  | 5.10 |      | 2.40 | 4.43 | 5.51  | 5.17  | 5.98  |
| 28 | 245161   | 5.00 | 4.80 | 4.80  | 3.80 | 3.40 | 4.50  | 5.10  | 5.30  | 5.10  | 5.10 | 4.50 |      | 5.84 | 6.19  | 6.24  | 6.53  |
| 29 | 245162   | 5.50 | 6.30 | 5.10  | 4.50 | 4.50 | 4.90  | 3.70  | 4.20  | 4.30  | 4.20 | 5.40 | 3.80 |      | 5.14  | 6.07  | 7.51  |
| 30 | Clemson  | 5.50 | 4.70 | 6.10  | 4.90 | 4.90 | 4.90  | 4.00  | 4.10  | 4.20  | 4.70 | 5.70 | 4.60 | 4.40 |       | 3.99  | 5.22  |
| 31 | SOH701   | 4.00 | 6.40 | 5.00  | 3.80 | 3.80 | 5.00  | 4.70  | 4.70  | 4.00  | 4.60 | 4.20 | 4.20 | 3.80 | 5.10  |       | 4.20  |
| 32 | SOH714   | 3.20 | 5.30 | 5.20  | 4.90 | 4.50 | 4.30  | 4.20  | 4.40  | 4.30  | 3.70 | 3.60 | 4.50 | 4.70 | 4.80  | 3.60  |       |

Note: Numbers in rows at top of table are designation of genotypes as listed in the first column of the table.

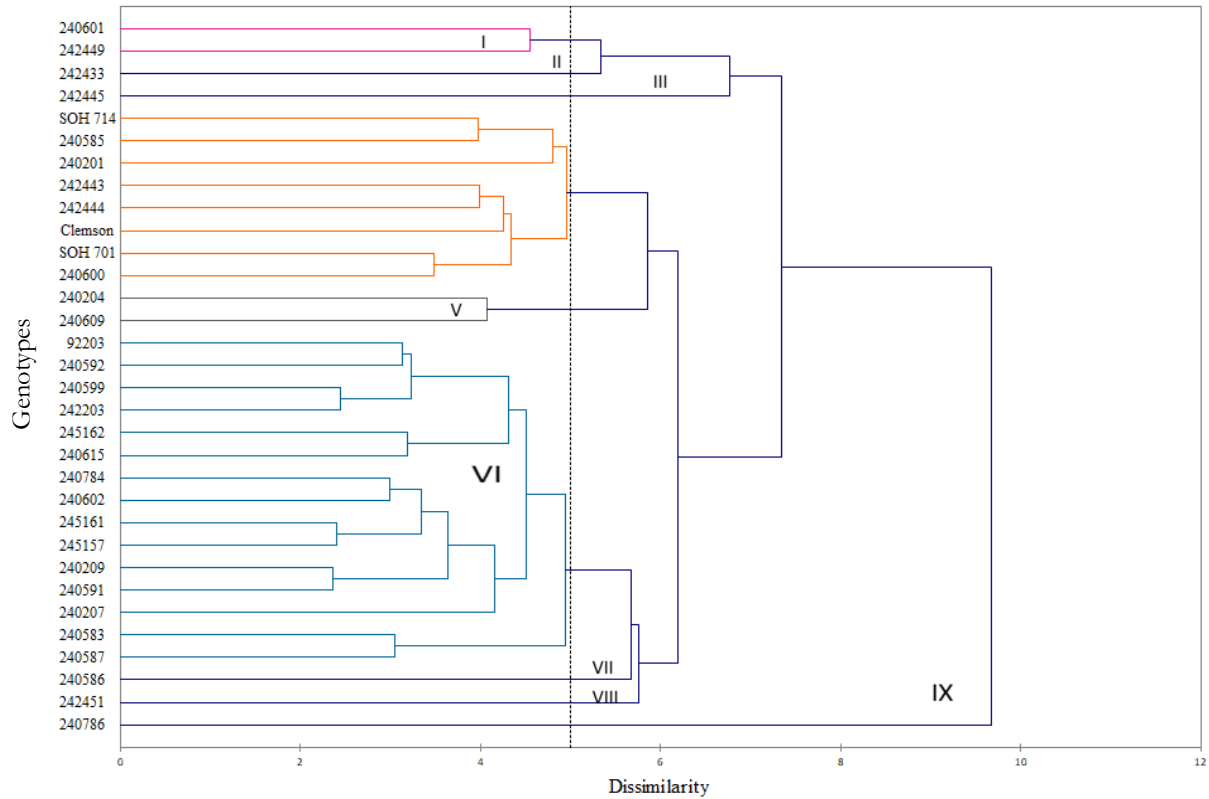


Figure 1. Dendrogram generated based on UPGMA clustering method among 32 okra genotypes based on 24 morpho-agronomic traits.

### 3.4. Diversity of Okra Genotypes based on SSRs Markers

#### 3.4.1. Characteristics of SSRs primers

A total of 121 alleles (fragments) were amplified by 19 primers of which 18(94.74%) primers were polymorphic amplified a total of 120 alleles. Among the 18 polymorphic primers, 14(77.78%) primers exhibited genetic diversity  $>0.67$ , 13(72.22%) had  $<0$  (negative) inbreeding coefficient and 14(77.78%) primers had

polymorphic information content (PIC) of 0.66 to 0.92 (Table 7). The amplified fragments (alleles) were greater than SSRs of *Medicago* used for okra diversity study (Sawadogo *et al.*, 2009) and the polymorphism information content was almost similar to values reported in 65 accessions of three okra species using the same 19 SSR markers (Schafleitener *et al.*, 2013).

Table 7. Nineteen SSRs primers size (bp), observed and effective alleles, heterozygosity and polymorphic information content.

| Locus        | ESR (bp) | OSR (bp) | N <sub>a</sub> | N <sub>e</sub> | H <sub>o</sub> | H <sub>e</sub> | F <sub>IS</sub> | PIC  | A (bp) | A <sub>f</sub> |
|--------------|----------|----------|----------------|----------------|----------------|----------------|-----------------|------|--------|----------------|
| AVRDC01      | 120–226  | 142–202  | 6              | 1.78           | 0.28           | 0.44           | 0.36            | 0.44 | 199    | 0.73           |
| AVRDC08      | 50–231   | 61–193   | 6              | 3.4            | 1              | 0.72           | –0.42           | 0.71 | 193    | 0.32           |
| AVRDC09      | 166–264  | 191–214  | 7              | 3.3            | 0.3            | 0.71           | 0.57            | 0.7  | 200    | 0.18           |
| AVRDC17      | 140–249  | 143–213  | 3              | 1.11           | 0.03           | 0.1            | 0.65            | 0.1  | 213    | 0.95           |
| AVRDC21      | 165–238  | 176–212  | 4              | 2.99           | 0.97           | 0.68           | –0.45           | 0.67 | 212    | 0.4            |
| AVRDC28      | 79–165   | 93–160   | 6              | 4.58           | 1              | 0.79           | –0.28           | 0.78 | 94     | 0.25           |
| AVRDC39      | 104–144  | 115–135  | 6              | 3.66           | 1              | 0.74           | –0.38           | 0.73 | 125    | 0.35           |
| AVRDC52      | 145–241  | 149–221  | 6              | 4.72           | 1              | 0.8            | –0.27           | 0.79 | 153    | 0.23           |
| AVRDC54      | 96–156   | 120–142  | 5              | 3.45           | 1              | 0.72           | –0.41           | 0.71 | 138    | 0.34           |
| AVRDC56      | 155–225  | 164–213  | 16             | 8.5            | 1              | 0.9            | –0.13           | 0.88 | 165    | 0.23           |
| AVRDC57      | 180–300  | 193–294  | 8              | 6.17           | 1              | 0.85           | –0.19           | 0.84 | 294    | 0.19           |
| AVRDC63      | 186–316  | 227–268  | 6              | 3.85           | 0.83           | 0.75           | –0.13           | 0.74 | 260    | 0.33           |
| AVRDC64      | 145–425  | 161–414  | 15             | 11.84          | 1              | 0.93           | –0.09           | 0.92 | 223    | 0.13           |
| AVRDC66      | 145–205  | 176      | 1              | 1              | 0              | 0              | –               | 0    | 176    | 1              |
| AVRDC70      | 155–241  | 174–197  | 3              | 2.93           | 1              | 0.67           | –0.52           | 0.66 | 174    | 0.39           |
| AVRDC77      | 180–280  | 209–239  | 4              | 1.88           | 0.59           | 0.48           | –0.27           | 0.47 | 209    | 0.7            |
| AVRDC78      | 175–265  | 207–234  | 8              | 3.02           | 0.19           | 0.68           | 0.72            | 0.67 | 210    | 0.52           |
| AVRDC86      | 77–260   | 80–250   | 6              | 1.94           | 0.44           | 0.49           | 0.1             | 0.48 | 85     | 0.7            |
| AVRDC89      | 124–199  | 145–174  | 5              | 3.88           | 1              | 0.75           | –0.35           | 0.74 | 158    | 0.33           |
| Overall mean | –        | –        | 6.4            | 3.89           | 0.72           | 0.64           | –0.08           | 0.63 | –      | –              |
| SE           | –        | –        | 0.8            | 0.6            | 0.09           | 0.06           | 0.09            | 0.06 | –      | –              |

Note: ESR = Expected size range; OSR = Observed size range; N<sub>a</sub> = Observed alleles; N<sub>e</sub> = Effective alleles; H<sub>o</sub> = Observed heterozygosity; H<sub>e</sub> = Expected gene diversity; F<sub>IS</sub> = Inbreeding coefficient; PIC = Polymorphic information content; A = Major allele; and A<sub>f</sub> = Major allele frequency.

### 3.4.2. Genetic distances and clustering of genotypes based on SSRs markers

The lowest Euclidean distance was 2.2 estimated between 242451 and 240591 and the highest was 7.10 estimated between 240784 and 240201 (Table 6). The genotypes had mean Euclidean distance of 4.65 with 0.77 standard deviation and coefficient variation of 16.51% calculated from 18 polymorphic SSR primers and 120 alleles. The dendrogram generated based on UPGMA clustering method from Euclidian distances matrix grouped the genotypes into seven distinct clusters. Cluster VII and VI consisted of 14(43.75%) and 9(28.13%) genotypes, respectively, whereas Cluster I and III were solitary consisted each one genotype. Cluster II and V consisted each two genotypes, while cluster IV constructed by three genotypes. SOH701 and SOH704 included in Cluster IV and VII, respectively, while Clemson spineless constructed solitary cluster (Figure 2). Similar to the morpho-agronomic traits, diversity analysis using SSRs primers also revealed the presence of wide range of genetic distances within okra genotypes obtained from Ethiopia than varieties introduced from other countries.

The genotypes grouped into same cluster had similarity for many alleles and genotypes grouped into different clusters had dissimilarity for one or more alleles to other genotypes in other clusters. Cluster analysis sequesters genotypes into clusters which exhibit high homogeneity within a cluster and high heterogeneity between clusters (Jaynes, 2003). The genetic differences of the okra genotypes were evident from SSRs data that grouped genotypes into seven clusters. The observed wide genetic difference might be due to geographic and environment variations where the genotypes were collected. The genotypes from southwestern Ethiopia collected at low altitudes (490 to 740 meters above sea level) and had geographical distance with genotypes from western and northwestern Ethiopia collected at >1000 meters above sea level except two genotypes (Table 1). The geographical isolation seems not much among the genotypes collected from western and northwestern Ethiopia, but the two geographic regions are separated by Abaya gorge. The reduced gene flow is associated with geographical, landscapes and ecologic factors as well as their combined effects (Nosil *et al.*, 2005; Crispo *et al.*, 2006; Wang *et al.*, 2013). Spatial isolation increases the genetic diversity between

populations (Pluess and Stocklin, 2004). The higher tendency of okra genotypes from each geographic region is to be grouped in same clusters (Wassu *et al.*, 2020). In addition, the crop produced from landraces in the regions might also contribute to the genetic distances of

the genotypes because of the absence of strong selection on phenotype (Mason and Taylor, 2015) and high frequencies of hybridization (Knowles and Richards, 2005) that reduce genetic divergence.

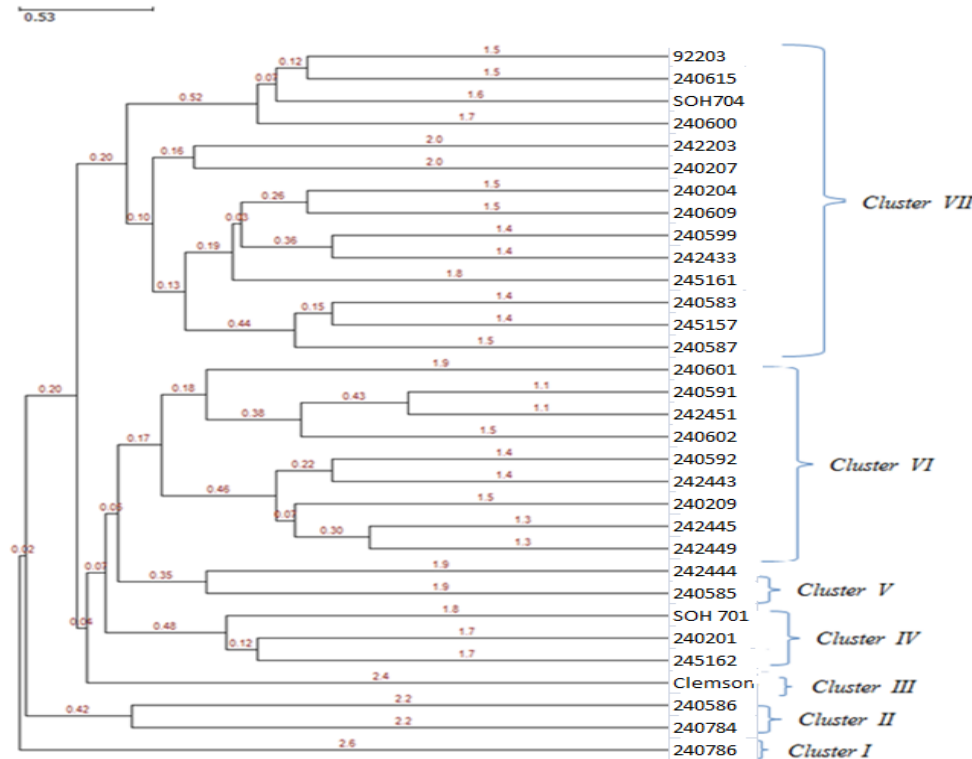


Figure 2. Dendrogram generated based on UPGMA clustering method among 32 okra genotypes based on 120 fragments amplified by 18 SSR primers.

### 3.5. Relationship of Genetic Distances Estimated from Morpho-agronomic Traits and SSRs

Regression analysis of the variance results revealed positive and highly significant ( $P = 0.00007$ ) relationship between Euclidian distances of 32 okra genotypes estimated for SSRs and 24 quantitative traits (Table 8). The significant and positive linear relationship between Euclidian distances estimated from SSRs and from 24 morpho-agronomic traits showed that both methods were viable and effective for the assessment and characterization of genetic diversity among okra genotypes.

It is common to use morpho-agronomic traits to assess the presence of genetic variation though the traits are limited in number and influenced by environment

(Fufa *et al.*, 2005). Many authors from different countries including from Ethiopia also reported the presence of genetic diversity among the varied number of okra genotypes based on phenotypic traits (Oppong-Sekyere *et al.*, 2011; Osawaru *et al.*, 2013; Mihretu *et al.*, 2014; Muluken *et al.*, 2015 & 2016; Younis *et al.*, 2015; Tesfa and Yosef, 2016). However, phenotypic variation may not always reflect the genetic diversity and information obtained from these traits alone not completely reliable to predict genetic variation that could be created in breeding programs (Fufa *et al.*, 2005; Sun *et al.*, 2015). Therefore, the authors agree with suggestion given by Schafleitener *et al.* (2013) that combining of SSRs with agromorphology data is the appropriate method to characterize okra germplasm collections to generate reliable diversity information.

Table 8. Summary of regression estimates and regression analysis of variance for Euclidian distances of 32 okra genotypes from SSRs and 24 quantitative traits.

| Source         | ED from SSRs and 24 quantitative traits |            |         |         |
|----------------|---|------------|---------|---------|
|                | SS                                      | MS         | F       | P       |
| Regression (1) | 9.2175                                  | 9.217523** | 16.0911 | 0.00007 |
| Residual (496) | 282.9796                                | 0.572833   |         |         |
| Total          | 292.1971                                |            |         |         |

| Summary of regression estimates for 496 pairs of ED from SSRs and 24 quantitative traits |          |          |          |          |          |          |  |
|--|----------|----------|----------|----------|----------|----------|--|
|  | Beta     | SE Beta  | B        | SE B     | t (493)  | P-level  |  |
| Intercept  | –        | –        | 4.317979 | 0.129564 | 33.32687 | 0.000000 |  |
| ED from SSRs and 24 quantitative traits  | 0.172584 | 0.044192 | 0.071451 | 0.018296 | 3.90531  | 0.000107 |  |

Note: ED = Euclidian distances; SS = Sum of square; and MS = mean square. \*\* refers to statistical significance at  $P < 0.01$ . Numbers in parenthesis are degrees of freedom.

#### 4. Conclusion and Recommendation

The results of this study have demonstrated wide variations among the okra genotypes for morpho-agronomic traits. The observed variations among the genotypes for days to flowering and duration of harvest, fruits characteristics, number of seeds per pod and tender fruit yield could be exploited to develop new varieties of the crop in breeding programs. Days to first flower bud, flower and fruit set, days to first fruit harvest, number and duration of harvest, number of primary branches, number and weight of fruit and dry pod per plant and number of seeds per pod had large contributions to the observed variability among genotypes. Thus, these traits could be used to study the variability of okra genotypes. That the growing season and genotype x environment interaction had significant effect on most of the morpho-agronomic traits suggests the importance of evaluating okra genotypes over seasons and locations to identify best adaptable and stably performing genotypes. The genetic distances estimated from morpho-agronomic traits and SSRs markers efficiently grouped the genotypes into 9 and 7 clusters, respectively. This suggests that both data from morpho-agronomic traits and SSRs markers generated reliable information on diversity of okra genotypes and one or both methods can be used for similar studies. However, it is better to use both methods in combination to identify diverse genotypes to develop okra varieties and/or conservation of okra germplasm in the country. The results of this study provide an insight into the extent of genetic diversity in the okra germplasm in Ethiopia. Thus, it is conclude that can be exploited in breeding programs to develop varieties through either

selection and/or hybridization. Further research has to be conducted to collect and evaluate other okra genotypes in the country and provide solid genetic base for improving the crop to enhance its production and productivity in the country.

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