

Phenotypic variations among okra (Abelmoschus esculentus (L.) Moench) genetic resources in Sudan

Ibrahim Mohamed El Tahir *

Agricultural Plant Genetic Resources Conservation and Research Centre, Agricultural Research Corporation, Wad Medani, Sudan

Abstract: A total of 366 accessions of okra (*Abelmoschus esculentus* (L.) Moench) obtained from the Agricultural Plant Genetic Resources Conservation and Research Centre (APGRC) in Sudan were characterized for a number of morphological characters using a descriptor list derived from the list published by the International Board for Plant Genetic Resources in 1984. Those accessions, which were collected from different regions of Sudan, were grown in the APGRC research farm in Wad Medani town in central Sudan during ten different seasons within the period between 2000 and 2019. Phenotypic variations were observed among and within the different accessions for plant, stem, leaf, inflorescence and fruit characters. Up to 59% of accessions were found to be heterogeneous for different traits. The descriptor states observed ranged from very rare in 5% or less of the accessions, to abundant in more than 90% of the accessions. Substantial phenotypic variation was observed for okra fresh fruits, the main organs used for food, in terms of shape, colour, pubescence and number of ridges. Accessions carrying fruits preferred in local or foreign markets were identified making them good candidates for further breeding to produce new cultivars for both markets. The cluster analysis resulted in 13 subclusters at a similarity level of 60%. When comparing the subclusters with collection sites, no direct relation was detected indicating that okra germplasm has been spreading all over the country resulting in diversified materials across different regions.

Keywords: Okra, genetic resources, characterization, Abelmoschus, Sudan

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Introduction

Okra (*Abelmoschus* spp.) is widely cultivated for its edible green fruits, which are harvested when immature and are famous for their slimy mucilage. It is widespread in tropical, subtropical and warm temperate regions. Common okra (*Abelmoschus esculentus* (L.) Moench) has been reported in the whole of tropical Africa, whereas West African okra (*Abelmoschus caillei* (A.Chev.) Stevels) is restricted to the humid and perhumid climates of Africa (Siemonsma and Kouamé, 2004).

Abelmoschus esculentus (usually 2n = 130) is probably an amphidiploid (allotetraploid), derived from *Abelmoschus tuberculatus* Pal & H.B.Singh (2n = 58), a wild species from India, and a species with 2n = 72chromosomes (possibly *Abelmoschus ficulneus* (L.) Wight & Arn. ex Wight) (Siemonsma and Kouamé, 2004). The exact origin of okra is unknown, however, one putative ancestor (*A. tuberculatus*) is native to Uttar Pradesh in North India, suggesting that *A. esculentus* originated in India. Another evidence is based on the plant's cultivation in ancient times and the presence of another putative ancestor (*A. ficulneus*) in East Africa, suggesting also northern Egypt or Ethiopia as the geographical origin of *A. esculentus* (Kumar *et al*, 2011).

Okra (*Abelmoschus* spp.) is the most popular traditional vegetable in Sudan, where both wild and cultivated types of okra are known. It is used in almost all parts of the country. It is cooked either after being dehydrated or as fresh pods (Mohamed, 1991). Some of the wild types seem to belong to the cultivated species *A. esculentus* while others belong to other species such as *A. ficulneus*. In Sudan, there is a late 19th-century record of its occurrence in the wild along the White Nile (Singh *et al*, 1975). Schippers (2002) reported that *A. ficulneus*

^{*}Corresponding author: Ibrahim Mohamed El Tahir (eltahir81@yahoo.com)

L. was found in Sudan and other regions of the Sahel (Mali, Chad) and East Africa, but more so in South and South East Asia. This wild species, of which both fruit and leaves are eaten in Sudan is one of the two ancestors of the common okra. Schippers (2002) also indicated that a range of primitive landraces could still be found in Sudan, where people collect fruit from wild plants for drying purposes. For example, the dehydrated okra locally called 'Waika', is collected from the wild from rain-lands in the central region and the southern Blue Nile, Kordofan and Darfur (Geneif, 1984).

Okra is cultivated in different parts of Sudan, where farmers depend almost completely on the use and production of traditional farmers' varieties. Apart from some introduced varieties such as Clemson Spineless and Pusa Swani, there are a number of farmers' varieties obtained from local types, which in many cases have names relevant to the localities, where they might have evolved and are usually produced. They include a spiny type called Khartoumia and other local varieties such as Karrari, Kassala, Medani, Sinnar and others (Ahmed and Mohamed, 1995).

Such variation in cultivated and wild okra varieties constitutes a wealth of indigenous genetic resources in Sudan that should be given the necessary attention to ensure their conservation against any possible threats, as well as to enhance their sustainable use. Therefore, germplasm collection efforts by the Plant Genetic Resources (PGR) Programme of the Agricultural Research Corporation (ARC) since the 1980s have resulted in the collection of more than 600 accessions of okra from different regions in Sudan with remarkable variation in plant and fruit characters. Seeds of such accessions are conserved in the genebank of the Agricultural Plant Genetic Resources Conservation and Research Centre (APGRC) of the ARC, which is located in Wad Medani town in Gezira state in central Sudan. Batches of seed samples are preserved under longterm seed storage conditions, which are realized in deep freezers adjusted at around -20°C, with seed moisture content within a 4-7% range. They are packaged in laminated aluminium foil packets, which are hermetically sealed before being deposited in the freezers.

This paper reports on efforts made and results obtained in Sudan under the APGRC for the enhancement of the use of okra genetic resources through morphological characterization from 2000 to 2019.

Materials and methods

Germplasm materials

Materials used and reported in this paper were genetic resources of okra (*Abelmoschus esculentus*) of which Sudan is a country of origin. 366 *A. esculentus* accessions, which were obtained by either direct collecting from different regions of the country or acquiring from other sources, such as okra breeders, were used in this work (Table 1, Supplemental Table 1). Passport data on

the accessions are documented in the genebank documentation system of the APGRC, and partially in the Genesys database, including georeferenced data of origin for some of them (Figure 1). All accessions were conserved in the APGRC seed genebank under long-term seed storage conditions. These accessions were grown and characterized in batches across ten different cultivation seasons during the period from 2000 up to 2019 as shown in Table 2.

Germplasm characterization

Samples of the 366 okra accessions were grown under full irrigation in the APGRC field for morphological traits characterization. The APGRC field is located within the research farm of the Gezira Agricultural Research Station at Wad Medani town, at latitude 14° 24' N and longitude 33° 29' E and altitude of 406.9m a.s.l. The climate of the area is hot semi-arid, and the soil is vertisol with clay content of 40–65%, and pH value ranging from 8 to 9.6, with less than 1% organic carbon, 300ppm total nitrogen and 406 to 700ppm total phosphorus (Ishag and Said, 1985).

Seed samples were obtained from the APGRC genebank and directly sown in the field in different seasons with sowing dates varied across seasons between the last week of June and the second week of July. Each accession was grown in a one-line plot on a ridge that was 80cm apart from others with in-row spacing of 20cm between plants. Each entry was represented by ten plants, at maximum, grown as one plant per hole. Routine cultural practices including manual removal of weeds, irrigation and raising of ridge sides were practised when required at intervals of around 7-15 days. Plants were nitrogen-fertilized using urea at a rate of 238kg of urea per hectare containing a total of 109.5kg of pure nitrogen. This total amount of urea was split into two doses; the first dose (119kg of urea per ha) was applied one month after sowing, while the second equal dose was applied one month later. Plants of all accessions were phenotypically characterized using 17 qualitative descriptors on plant, stem, leaf, inflorescence and fruit traits derived from the International Board for Plant Genetic Resources descriptor list for Abelmoschus (Charrier, 1984) (Supplemental Table 2). All traits were fully described in the field by recording all descriptor states that were observed within each accession.

Statistical analysis

The frequency level of different descriptor states was calculated as the percentage of accessions showing homogeneously only the same specific descriptor state within the 366 accessions characterized. Accessions showing different states for a descriptor were considered heterogeneous for that specific descriptor and their frequency percentages were calculated as well. Accordingly, the descriptor states were considered to be occurring at different levels including very rare, rare, moderate, common and abundant. They were categorized as very rare when occurring with a frequency level of 5% or less, rarely when occurring with a frequency level > 5% and $\le 30\%$, moderately when occurring with a frequency level > 30% and $\le 60\%$, commonly when occurring with frequency levels > 60% and $\le 90\%$ and abundantly when occurring with frequency levels > 90%.

Multivariate analysis was run on the data obtained through the hierarchical cluster analysis with complete linkage using the software GenStat Twelfth Edition (GenStat Release 12.1).

Table 1. Number of accessions collected from differentgeographical regions in Sudan and used in this study.

Administrative state	Total accessions
South Kordofan (including the	73
present South Kordofan and West	
Kordofan states)	
West Darfur	50
Northern State	38
West Kordofan	31
South Darfur	27
Blue Nile	25
Red Sea	24
Gedarif	21
North Kordofan	17
Sinnar	17
Kassala	13
River Nile	11
Central Darfur	6
Khartoum	5
White Nile	5
Gezira	4
Unknown	29
Total	366

 Table 2. Number of okra accessions characterized in different cultivation seasons

Season of cultivation	Total number of
	accessions characterized
2000-2001	43
2006–2007	86
2007–2008	35
2008–2009	39
2009–2010	33
2011-2012	16
2013–2014	15
2015–2016	12
2017–2018	33
2018–2019	54
Total	366

Results

A total of 60 descriptor states were observed for the 17 descriptors covering plant, stem, leaf, inflorescence and

fruit characters across the 366 okra accessions studied (Supplemental Table 3). A total of 150 accessions (41%) were observed to be homogeneous for all descriptors, while the remaining 59% (216 accessions) were heterogeneous for one or more descriptors. However, the level of heterogeneity for each descriptor, calculated as the frequency of accessions showing different descriptor states for the same descriptor within the same accession, ranged between 3% being the lowest level of heterogeneity for the descriptor of fruit peduncle length, and 34% being the highest level of heterogeneity for (Table 3).

The frequency level of different descriptor states ranged between less than 1% and 100% as shown in Table 3. Some of the descriptor states were observed to be abundant in more than 90% of the accessions characterized such as the linear shape of epicalyx segments (91%), or the yellow flower colour (100%). On the other hand, some descriptor states were very rarely observed in only 5% or less of the accessions such as the procumbent general aspect of plant (2%), purple stem colour (3%), pendulous fruits on main stem (< 1%) or fruits that were red or green with red patches (< 1%, and 4% respectively). Otherwise, phenotypic variations were observed at different levels across the different plant organs of the okra accessions.

Phenotypic variations related to plant vegetative characters

Variations were observed in the vegetative characters that included general plant aspect, branching, leaf and stem characters, as shown in Table 3.

General plant aspect

Erect, medium and procumbent plant aspects were observed. Half of the characterized accessions had medium aspect, while 23% had erect plants, and the procumbent aspect was observed very rarely in only 2% of the accessions. The rest of the accessions (25%) were heterogeneous for this descriptor.

Branching

Different descriptor states of branching were observed among the okra accessions studied including orthotropic, medium and strong branching. While medium branching was observed in the majority of the accessions with a frequency of 51%, orthotropic and strong branching were rarely observed (19% of the accessions for either). The rest of the accessions were heterogeneous for this descriptor.



Figure 1. Collection sites in Sudan of some okra accessions described in this paper, for which georeferenced data was available from APGRC.



Figure 2. Descriptors for leaf shapes used for characterization purposes (Charrier, 1984)

Table 3. Phenotypic variations observed in the 366 characterized okra accessions. Occurrence level: Very rare (\leq 5%), rare (> 5%, \leq 30%), moderate (> 30%, \leq 60%), common (> 60%, \leq 90%), and abundant (> 90%).¹⁾Leaf shape and some fruit characters are depicted in Figure 2 and Figure 5.

Descriptor	Occurrence level of each descriptor state and its frequency percentage					
Descriptor	Abundant	Common	Moderate	Rare	Very rare	Heterogeneous
General aspect			Erect (50%)	Medium (23%)	Procumbent (2%)	25%
Branching			Medium (51%)	Orthotropic (19%)		11%
				Strong (19%)		
Stem pubescence			Slight (45%)	Glabrous (22%)		26%
				Conspicuous (7%)		
Stem colour			Green (49%)	Green with red patches (22%)	Purple (3%)	26%
Leaf shape ¹⁾				3 (7%)	5 (< 1%)	34%
				4 (24%)	6 (< 1%)	
				7 (24%)	8 (1%)	
				10 (7%)	9 (2%)	
Leaf colour			Green with red veins (48%)	Green (24%)		28%
Number of epicalyx segments			8–10 (42%)		5–7 (2%)	16%
			> 10 (40%)			
Shape of epicalyx segments	Linear (91%)				Lanceolate (9%)	
Petal colour	Yellow (100%)					
Red colouration of petal base		Both sides (76%)		Inside only (17%)		7%
Position of fruit on main stem		Erect (85%)		Horizontal (6%)	Pendulous (< 1%)	9%
Fruit colour			Green (47%)	Yellowish green (23%)	Green with red patches (4%) Red (< 1%)	26%

Continued on next page

Table 3 continued						
Descriptor	Occurrence level of each descriptor state and its frequency percentage					
Descriptor	Abundant	Common	Moderate	Rare	Very rare	Heterogeneous
Fruit length at maturity			8–15 cm (40%)	< 8cm (11%)		26%
				> 15cm (23%)		
Length of peduncle		1–3 cm (84%)		> 3cm (13%)		3%
Fruit shape ¹⁾				1 (21%)	2 (< 1%)	25%
				3 (23%)	5 (< 1%)	
				4 (16%)	8 (< 1%)	
				6 (6%)	10 (< 1%)	
					11 (< 1%)	
					12 (< 1%)	
					14 (5%)	
					15 (< 1%)	
Number of ridges per fruit		5–7 (70%)		< 5 (9%)	> 10 (1%)	10%
				8–10 (10%)		
Fruit pubescence				Downy (29%)		27%
				Slightly rough (17%)		
				Prickly (27%)		

State	Total accessions	No. of subclusters
Northern State	38	10
South Darfur	27	10
Gedarif	22	9
South Kordofan	73	9
Red Sea	24	7
North Kordofan	17	6
Sinnar	17	6
Blue Nile	25	5
West Darfur	50	5
White Nile	5	4
Gezira	4	3
Kassala	13	3
River Nile	11	3
Central Darfur	6	1
Khartoum	5	1
Unknown	29	9
Total	366	

Table 4. Number of subclusters at similarity level of 60% among which accessions from each state are distributed.

Leaf characters

Leaf shape was among the characters with maximum variations recorded in the okra accessions characterized. Eight descriptor states were observed in terms of leaf shapes. Consequently, all descriptor states observed for shapes of leaf were found to be either very rarely or rarely occurring with frequency levels of less than 1% and up to 24%. However, the most common leaf shapes were shape 4, with a five-lobed leaf with smooth margins (24%); and shape 7, with a five-lobed leaf with non-smooth undulating margins (Figure 2) (24%). On the other hand, the least occurring leaf shapes were shapes 5, 6, 8 and 9, which were very rarely occurring at frequencies of less than 1% to 2%. The rest of the accessions (34%) were heterogeneous for this descriptor.

Leaf colours were green (24%) and green with red veins (48%), in the accessions that were homogeneous for this character. The rest of the accessions were heterogeneous for this descriptor.

Stem characters

Stems of most accessions were either green or green with red patches with frequencies of 49% and 22%, respectively, among the homogeneous accessions characterized. On the other hand, purple stems were very rarely observed in only 3% of the accessions.

Stems were either glabrous, slight or with conspicuous pubescence. Most of the accessions were either glabrous (22%) or had slight hairs on their stems (45%). Otherwise, only 7% of the accessions had conspicuous hairs, while the rest of the accessions were heterogeneous for this character.

Variations in inflorescence characters

The only characters that were abundantly dominating in the studied germplasm were the linear shape of epicalyx segments, and the yellow flower colour, which were occurring in 91% and 100% of the accessions, respectively.

Red colouration was commonly found at both sides of the petal base in the majority of the accessions (76%), while only 17% of the accessions produced flowers with red colouration only inside the petal base. The rest of the accessions were heterogeneous for this character.

Forty two percent of accessions had between 8 and 10 epicalyx segments, and 40% had more than 10 segments with a moderate level of occurrence. On the other hand, 5–7 segments were observed very rarely, in only 2% of the accessions. The rest of the accessions were heterogeneous.

Variations in fruit characters

Variations were observed among the okra accessions on different fruit-related characters (Table 3).

Position of fruit on main stem

Erect position of fruits on main stem was a common character observed in the majority of the accessions at a frequency of 85%. The horizontal and pendulous positions of fruits on main stems were rarely or very rarely observed, with frequencies of 6% and less than 1%, respectively. The rest of the accessions were heterogeneous for this character.

Fruit colour

The majority of fruits produced were green (47% of the accessions) or yellowish green (23%). Fruits that were red or green with red patches were very rarely produced by fewer than 1% and 4% of the accessions, respectively. The rest of the accessions were heterogeneous for this character. Exceptionally, it was observed that remarkable greener ridges were observed on green or yellowish green fruits in some accessions (Figure 3).

Fruit length at maturity

Forty percent of the characterized accessions produced fruits that were 8–15 cm long when mature. Fruits shorter than 8cm or longer than 15cm were produced by 11% and 23% of the accessions, respectively (Figure 4). The rest of the accessions were heterogeneous for this character.

Length of peduncle

The majority of the accessions had fruits with peduncles that ranged between 1 and 3cm long in 84% of the accessions, while 13% of the accessions were producing fruits with peduncles longer than 3cm or were heterogeneous for this character (3%).

Fruit shape

Fruit shape was among the characters with maximum variation in terms of the number of descriptor states observed within the germplasm collection studied,



Figure 3. Variations in fruit colour as observed on some accessions.



Figure 4. Variations and frequency percentages of mature fruit lengths among the characterized accessions.

recording 12 descriptor states at different frequencies that ranged between less than 1% and 23%. The most common fruit shapes observed in 16–23% of the accessions were shapes 1, 3 and 4, which are characterized by elongated ridged capsules that were slender and moderately curved in shape 1, but straight or very slightly curved for shapes 3 and 4, with a wider base in shape 4 (Figure 5). Nine fruit shapes were observed very rarely, occurring in less than 1% to 6% of the accessions, while 25% of the accessions were heterogeneous for this descriptor (Figure 5).

Number of ridges per fruit

The number of ridges per fruit ranged between five and seven in the majority (70%) of the accessions characterized. Fruits with fewer than five ridges or with eight to ten ridges were rarely observed, in 9% and 10% of accessions, respectively. Fruits with more than ten ridges were very rarely observed (less than 1%). Ten percent of accessions were heterogeneous.

Fruit pubescence

Most of the accessions characterized were homogeneous for fruit pubescence producing either downy, slightly rough or prickly pubescent fruits occurring at rare or moderate levels with frequencies ranging between 17% and 29%.

Results of cluster analysis

Cluster analysis resulted in a clustering pattern composed of two major clusters at similarity level of 30%. These major clusters were further grouped into 13 subclusters at a level of similarity of 60% including 4 subclusters under the first major cluster, and 9 subclusters under the second (Figure 6). When comparing the clustering pattern with the collection sites of the accessions, no direct relation was detected (Table 4). The only exceptions were the accessions from Central Darfur and Khartoum states as they clustered in only one group indicating a limited variation among them. However, this could be attributed to the limited number of accessions obtained and studied from these two states (six from Central Darfur and five from Khartoum).

Discussion

The okra accessions described in this study were collected and obtained from different regions and sources in Sudan. The majority of the accessions were heterogeneous, which is normal in a crop for which farmers' varieties are mainly used, as is the situation in



Figure 5. Descriptor states of fruit shapes (Charrier, 1984), and some of the observed fruit shapes and their frequency percentages as shown by some accessions.



Figure 6. Dendogram of clustering patterns of 366 characterized okra accessions showing the two major clusters (A and B) and the four subclusters under A (A1-A4) and the nine subclusters under B (B1-B9).

Sudan. Hamon and Van Sloten (1989) indicated that a homogeneous accession is a rare occurrence in okra as it is reproduced by seeds and is not strictly autogamous.

This study has shown that the Sudanese okra germplasm is rich in terms of variation for a number of morphological traits. Among them are traits significant for yield and quality of the okra fruits, which are consumed by people. A considerable number of the okra accessions in this study had erect growth habit and medium to strong branching, which could be indication for high yielding potential, as described in a similar study that recorded strong erect stems and dense branching for *Abelmoschus* species at production sites with extended harvest throughout the year (Omonhinmin and Osawaru, 2005). It is interesting to note from this study that fruit shape was among the characters with the highest level of observed variation in Sudan with 12 descriptor states out of the 15 recommended in the IBPGR descriptors for *Abelmoschus* (Charrier, 1984). This was similar to the results reported by Oppong-Sekyere *et al* (2011) on okra germplasm from Ghana, where fruit shape showed the greatest diversity among the okra accessions, from short and triangular to long straight or long curved. However, only three shapes were the most frequently observed in this collection, which were actually closely similar to each other as all of them were of elongate fruit type with variation in thickness and curvature. In fact, this is the type of okra fruits that are most commonly consumed and sold in the vegetable markets in the country. Such phenomenon indicates the trend among the okra farmers in Sudan to select for this type of elongate fruits to meet the market demand and the consumers' preference.

A number of characters observed seem to be very rarely or rarely present in the described collection indicating a higher risk to lose them. Among those are characters such as procumbent aspect of plant, purple stems, pendulous fruits on main stems, fruit that were red or green with red patches, and a number of fruit shapes other than the elongate ones. More attention should be paid to conserve accessions carrying such characters in order to preserve, multiply and evaluate them for traits other than those related to the preferred fruit quality and yield potential such as resistance to biotic and abiotic factors. However, some of these rare characteristics were also observed with similar frequency in a recent study conducted in neighboring Ethiopia where only 19.44% of accessions had red or purple stem colour (Temam et al, 2021).

The majority of the accessions produced fruits that were either green or yellowish green, while red fruits or green fruits with red patches were rarely produced in about 5% of the accessions. This also refers to the tendency of the okra farmers in Sudan to select for greenish fruits that are usually preferred by the consumers. On the other hand the results of the study in Ethiopia by Temam et al (2021) showed that fruits that were green with red patches were the most highly represented in their collection at a frequency of 55.56%. While Sudanese consumers do not tend to use green fruits with red patches, it seems this fruit colour is preferred by the consumers of okra in at least some regions of Ethiopia. On the opposite the Ethiopian consumers may not like fruits that were yellowish green as only 2.78% of the characterized Ethiopian accessions produced such type of fruits, while 23% of the Sudanese accessions in the present study had yellowish green fruits.

Okra consumers in Sudan also tend to prefer the spiny okra fruits, as they are locally believed to be more slimy and tasty than others. The results obtained from this study showed that a considerable number of characterized accessions (44%) had spiny texture by being either prickly or slightly rough. However, downy fruits with smooth texture were also recorded for a considerable number of accessions (29%) indicating the potential of the okra germplasm in Sudan for use to produce varieties that are more likely to be suitable for exportation outside the country where downy green fruits seem to be more preferred.

The abundant occurrence of linear epicalyx segments in the collection described with very rare cases of lanceolate segments and the full absence of triangular segments confirm that the accessions characterized belong to the species of the common okra *Abelmoschus esculentus* that is common in east Africa. In contrast to this the species *A. caillei*, which is known as the West African okra, is more common in West Africa. As mentioned by Siemonsma and Hamon (2004) *A.* *esculentus* differs in several respects from *A. caillei*, but the epicalyx offers the best discriminating characteristic: the width of the epicalyx segments is 4–13mm in *A. caillei* and 0.5–3mm in *A. esculentus*.

Generally, the okra genetic resources from Sudan have considerable phenotypic variations among them as shown by the clustering pattern of the accessions described in this study reaching to 13 sub-clusters at 60% level of similarity. The variation is also detected among the okra accessions collected from each of the geographical states as they were mostly clustering in more than one group reaching to 10 sub-clusters in some states. Such trend of variations in okra genetic resources means that okra germplasm has been spreading in Sudan from different sources resulting in diversified phenotypes all over the country and in each of the different regions.

Conclusion

This study has shown that the germplasm collection of okra from Sudan held by the APGRC, which is mostly composed of farmers' varieties, is diverse in a number of characters including those important for yield and quality for both local and foreign markets. Such diversity in this collection makes it highly promising for breeding for improved okra cultivars through purification, selection and evaluation for superior lines. However, more germplasm collection of okra genetic resources is needed with emphasis on regions and geographical pockets that are poorly represented in the materials collected and studied so far. Further characterization for morpho-agronomic traits is also necessary as well as evaluation of such germplasm for desirable traits including resistance and adaptability to biotic and abiotic stresses.

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Conflict of interest statement

The author declares that there is no conflict of interest.

Supplemental Data

- Supplemental Table 1: List of all okra accessions characterized, their sites of collection and characterization seasons
- Supplemental Table 2: Okra descriptors and descriptor states used in the study
- Supplemental Table 3: Characterization results recorded on the different okra accessions

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