Journal of Tropical Biology and Conservation 16: 197–211, 2019 ISSN 1823-3902
E-ISSN 2550-1909

Short Notes

Assessing the Relatedness of *Abelmoschus* Accessions using Morphological Characters

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Abstract
Character analysis of Okra (*Abelmoschus* [Medik.] species, Malvaceae) accessions was carried out using morphological data to evaluate their genetic distinction and relatedness. Seeds of five *Abelmoschus* accessions (NG/MR/01/10/002, A.E 3, NG/MR/MAY/09/009, NGAE-96-0065 and NG/OA/05/12/160) were obtained from the Gene Bank of National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria. Based on the International Board for Plant Genetic Resources standard descriptors for Okra, 16 qualitative morphological characters were selected based on their relevance to *Abelmoschus* breeding, crop distinction, utilization and conservation. The five accessions present significant differences with two of the accessions (NG/MR/MAY/09/009 and NG/OA/05/12/160) closely related and other three (NG/MR/01/10/002, A.E 3 and NGAE-96-0065) closely related too. Accessions NG/MR/01/10/002, A.E 3 and NGAE-96-0065 had medium or intermediate growth habit while accession NG/MR/MAY/09/009 and NG/OA/05/12/160 shows erect growth habit. General aspect of the stem, nature of branching, fruit pubescence, fruit shape, position of the fruit on the main stem, leaf shape, fruit colour, and fruit length at maturity had the most effect on observed relationship between the accessions. Scatter plots derived from the principal component analysis suggest moderate tendency of grouping with the genus where two distinct clusters were obtained from the dendrogram. Together, these results suggest that the five okra accessions may be the descendants of the two commonly cultivated *Abelmoschus* species in Southern Nigeria (i.e. *A. esculentus* and *A. caillei*).

Keywords: Okra (*Abelmoschus* species), Genetic diversity, Plant Conservation, Character analysis, Morphological data

Received 16 April 2019
Reviewed 08 July 2019
Accepted 22 July 2019
Published 15 October 2019

Introduction

Okra (*Abelmoschus* [Medik.] species) belongs to the family Malvaceae and order Malvales. Angiosperm Phylogeny Group (2009) recognized it as a monophyletic group. The group contains economically important crops used for food, fibre vegetable oil, horticulture, timber, medicine, etc. Common examples include jute (*Corchorus*) and cotton (*Gossypium*). Members of this group are cultivated throughout the tropics and subtropics in home gardens as well as on large commercial farms (Osawaru et al., 2014). Their cultivation is influenced by environmental conditions. For instance, Shahid et al. (2011) reported that increasing salinity causes a decrease of germination percentage, shoot and root length, plant height, pod weight, pod length, photosynthesis rate, and stomatal conductance. *Abelmoschus* species is a multipurpose and functional food crop with increasing importance especially with regard to its nutritional, medicinal, and industrial value (Reddy et al., 2013; Ogwu et al., 2016a). Osawaru et al. (2011) reported that although about 60% of okra grown is for subsistence as fresh vegetable, okra parts and products remain the subject of major economic activities in West Africa. Okra production plays a significant role in the economy, hence more attention should be given to the selection of high yielding cultivars to maximize its socio-economic potential (Adeoluwa & Kehinde, 2011; Kumar et al., 2010).

Abelmoschus species have diverse morphological characteristics in Africa and Asia. Some are annual herbs, perennial shrubs or trees and produce a characteristic mucilaginous substance (Edwin et al., 2006). In spite of its high acceptability among growers, consumers and their wide genetic pool variability, optimum productivity is still lagging (Das et al., 2012). Eshiet and Brisibe (2011) opined that cultivated okra rarely reach their maximum yield potential due to several constraints including poor investments in breeding programmes aimed at enhancing their yield in the field. Nonetheless, okra has huge potential for enhancing livelihoods in urban and rural areas as it offers a possible route to prosperity for small-scale and large-scale producers as well as to all those involved in the okra value chain, including women producers and traders (Kumar et al., 2010). The first step towards this goal is to address their taxonomy. Taxonomy of *Abelmoschus* has a complex history with uncertainty in the generic and specific status because of the inconsistent treatment for some *Abelmoschus* species (Patil et al., 2015). Characterization studies will help highlight distinct descriptive features that may be explored in plant breeding. The success of a breeding programme depends on the variability of the initial material as well as available knowledge base of the genetic system, which is important for devising an efficient selection programme through the use of a suitable mating design

(Reddy et al., 2013). More so, characterization of genetic resources is an essential first step in any crop improvement (Das et al., 2012) and conservation programme (Ogwu et al., 2014; Osawaru and Ogwu, 2014). Characterization and quantification of genetic diversity within and among closely related crop varieties are essential for their rational use (Adeoluwa and Kehinde, 2011). Diversity based on phenotypic and morphological characters usually varies with environments; hence the evaluation of traits may require their cultivation to maturity prior to identification and characterization (Adeoluwa and Kehinde, 2011). Through characterization, the nature of the relationship between and among plant species can be highlighted with the help of description keys (Osawaru et al., 2015a). This is necessary since the selection of superior genotypes may be based on outward appearance (phenotype), which is subject to variation due to fluctuating environmental factors (Adeoluwa and Kehinde, 2011).

Reproductive and vegetative morphology of *Abelmoschus* species is known to be variable but the patterns of this variation and their relevance in the crop distinction, breeding, germplasm utilization and conservation have not been sufficiently addressed (Patil et al., 2015, Osawaru et al., 2015b; Ogwu et al., 2016). This study aims to characterize *Abelmoschus* accessions based on morphological data with a view to assess their genetic relationship, highlight the key descriptive character and to promote the conservation and sustainable utilization of the germplasm.

Materials and Methods

Material collection and cultivation: Seeds of five accessions of *Abelmoschus* species (NG/MR/01/10/002, A.E 3, NG/MR/MAY/09/009, NGAE-96-0065 and NG/OA/05/12/160) were obtained from the Gene Bank of the National Centre for Genetic Resources and Biotechnology, Ibadan, Nigeria.

The seeds were grown on an experimental field beside the Botanical Garden, University of Benin, Nigeria using randomized complete block design with three replicates per accession. Fifty pre-soaked (for 24 h) seeds (10 from each accession) were sown directly in the soil (3 – 5 cm deep) per plot with a spacing of 40 × 30 cm (row to row and plant to plant) and covered with top soil. Seedlings were thinned to three plants per stand two weeks after germination. The physicochemical and microbial characteristics of the plot have been reported by Osawaru et al. (2013a; 2013b); Osawaru and Ogwu (2014b); Ogwu and Osawaru

(2015b). Standard agronomic practices such as weeding and watering were adopted from Remison (2005).

Morphological characterization: Data were collected on morphological characters using standardized descriptors for o (IBPGR, 1991). Based on this descriptor, 16 qualitative characters were measured.

Table 1. Code used in morphological characterization.

S/N	Parameter measured	Parameter key	Character state
1	General aspect of stem	GAS	3=erect, 5=medium, 7=procumbent
2	Stem colour	StC	1=green, 2=green with red patches, 3=purple
3	Stem pubescence	SPu	3=glabrous, 5=slight, 7=conspicuous
4	Nature of Branching	Brc	3=orthotropic stem only, 5=medium, 7=strong
5	Leaf shape	LSh	From types 1 to 11
6	Leaf colour	LCl	1=green, 2=green with red veins, 3= red
7	Petiole colour	PtC	1=green, 2=green with red veins, 3=purple
8	Flowering span	FSp	1=single flowering, 2=grouped flowering
9	Petal colour	PCl	1=cream, 2=yellow, 3=golden
10	Fruit colour	FCl	1=yellowish green, 2=green, 3=green with red patches, 4=red
11	Position of fruit on main stem	PFS	3=erect, 5=horizontal, 7=pendulous
12	Fruit shape	FSh	From types 1 to 15
13	Fruit pubescence	FPu	3=downy, 5= slightly rough, 7=prickly
14	Length of peduncle	LPe	1=from 1 to 3cm, 2=more than 3cm
15	Fruit length at maturity	FLM	1=less than 7cm, 2= from 8 to 15cm, 3= more than 15cm
16	Seed shape	SSh	1=round, 2=reniform

Statistical analysis: Multivariate statistical analyses were employed to assess the genetic relationship among accessions as suggested by the morphological data. Data collected were analyzed using SPSS (version 20.0) and PAST (paleontological statistics, version 1.34). Principal Component Analysis (PCA), Scatter Plot and Single Linkage Cluster Analysis (SLCA) were used to determine the extent of genetic variation and percentage similarities within and between accessions. Eigen-values, factor scores and scatter diagram obtained from PCA were used to determine the relative discriminative power of axes and their associated characters. The dendrogram was generated from the SCLA to display position of accessions and their distance similarity.

Result

The results are shown in Tables 2 - 4 and Figures 1 and 2.
The results of morphological trait assessment of the five accessions of *Abelmoschus* species is presented in Table 3.

The principal component analysis was conducted for 16 qualitative morphological characters among the five accessions of *Abelmoschus* spp. This shows that only one of the 16 principal component axes had eigenvalues greater than 2.43 as Joliffe cut-off standard value according to Joliffe (2002).

The eigenvalue tells the importance of each principal component axes and its contribution in explaining the variability in characters of five accessions of *Abelmoschus* spp. The PCA eigenvalue includes: PC 1 (10.54), PC 2 (2.36), PC 3 (0.58) and PC 4 (0.43); 0.40 was taken as the standard.

Table 2. Qualitative traits that varied among the five accessions of *Abelmoschus* species

S/N	Accession Parameter Measured	NG/MR/01/10/002	A.E 3	NG/MR/MAY/09/009	NGAE-96-0065	NG/OA/05/12/160
1	GAS	5	5	3	5	3
2	StC	2	2	2	2	2
3	SPu	3	3	3	3	3
4	Brc	5	7	3	7	3
5	LSh	4	4	4	4	4
6	LCl	2	2	2	2	2
7	PtC	2	2	2	2	2
8	FSp	1	1	1	1	1
9	PCl	3	3	2	2	2
10	FCl	2	3	2	2	3
11	PFS	7	7	3	7	3
12	FSh	4	4	4	4	1
13	FPu	5	7	3	5	3
14	LPe	1	2	1	2	1
15	FLm	2	2	1	2	2
16	SSh	2	2	2	2	2

Key:
GAS- general aspect of stem, **SP**- stem pubescence, **Br**-branching **StC**-stem colour **LC**-leaf colour, **PC**- petal colour, **PFMS**-position of fruit on main stem, **FC**- fruit colour, **FLm**- fruit length at maturity, **LP**-length of peduncle, **FSh**- fruit shape, **FP**- fruit pubescence, **SSh**- seed shape, **FSp**- flowering span **LSh**- leaf shape, **PtC**-petiole colour

Table 3. Principal Component Analysis (PCA) of 16 qualitative morphological characters among five accessions of *Abelmoschus* species

Characters code	PC 1	PC 2
GAS	1.04	0.08
SPu	2.26e-17	3.15e-17
Brc	0.59	0.27
StC	-8.42e-20	8.39e-18
LCl	-3.12e-22	1.74e-19
PCl	0.11	0.03
PFS	0.32	0.08
FCl	-0.02	0.29
FLM	0.06	0.23
LPe	0.13	0.10
FSh	0.30	-0.59
FPu	0.48	0.26
SSh	0	0
FSp	0	0
LSh	-0.30	0.59
PtC	0	0
Eigenvalue	10.54	2.36
% Variance	75.80	16.97

Key:
GAS- general aspect of stem, **SPu**- stem pubescence, **Brc**-branching **StC**-stem colour **LCl**-leaf colour, **PCl**-petal colour, **PFS**-position of fruit on main stem, **FCl**- fruit colour, **FLM**- fruit length at maturity, **LPe**-length of peduncle, **FSh**- fruit shape, **FPu**- fruit pubescence, **SSh**- seed shape, **FSp**-flowering span **LSh**-leaf shape, **PtC**-petiole colour **PC**- Principal component.

The loading of each character on the different principal component axes is used to assess their relative contribution in showing variation. The following characters was heavily loaded along PC axis 1; GAS (1.04), Brc (0.59), FPu (0.48), FSh (0.30) and PFS (0.32) while LSh (0.59), FCl (0.29) and FLM (0.23) were loaded along PC axis 2.

Table 4. The Standardized Principal Component scores

Accession	PC 1	PC 2	PC 3	PC 4
NG/MR/01/10/002	**1.077**	-0.576	0.588	0.966
A.E 3	**3.326**	0.883	0.649	-0.711
NG/MR/MAY/09/009	-2.450	-2.201	0.058	-0.428
NGAE-96-0065	**2.284**	0.032	-1.245	0.063
NG/OA/05/12/160	-4.237	**1.862**	-0.044	0.110

Key:
PC - Principal component.

The standardized principal component scores of the various accessions suggest that the first two principal component axes account for 92.77 % of the variance from the 16 qualitative morphological parameters (axes). PCA 1 loaded characters are possessed by accession A.E 3, NGAE-96-0065 and NG/MR/01/10/002 respectively. PCA 2 loaded characters are best possessed by accession NG/OA/05/12/160 only.

Accession A.E 3, having seen to have a high PCA score in PCA 1 could be well distinguished by characters in PCA 1. For instance, the general aspect of stem, branching and fruit pubescence are seen to be high in accession A.E 3 and NGAE-96-0065 respectively and highly loaded in PCA 1. These characters are outlined in Table 3.

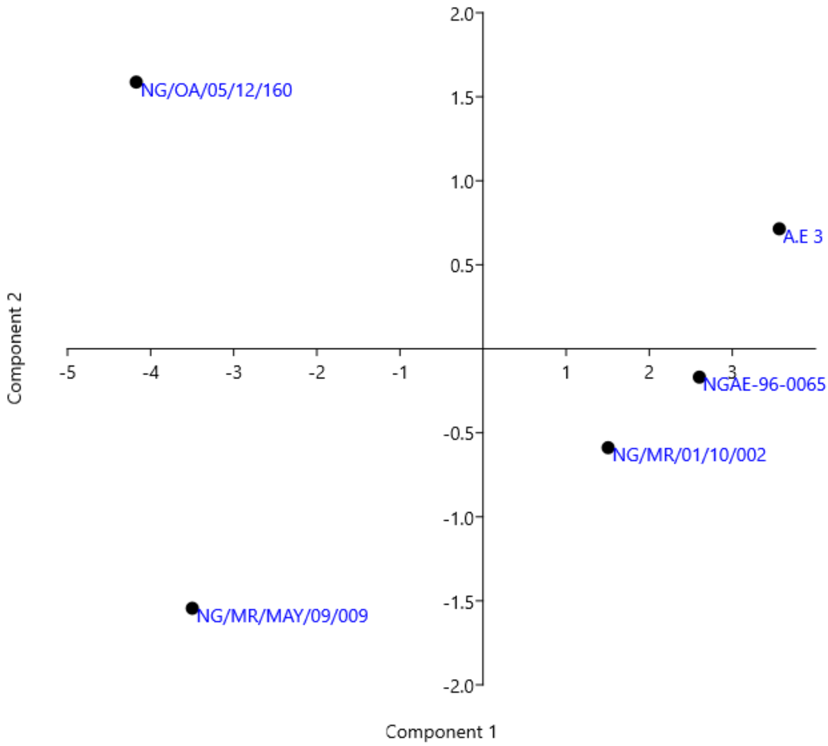

Figure 1: PCA scatter diagram produced by plotting the first PC against the second PC for 16 qualitative morphological characters of five accessions of *Abelmoschus* species

The scatter plots derived from the PCA also show the grouping tendency of the accession studied by grouping accessions NG/MR/MAY/09/009 and NG/OA/05/12/160 together and accessions NG/MR/01/10/002, A.E 3 and NGAE-96-0065 in the other component.

The clustering pattern of the dendrogram also suggests the existence of two groups like *A. caillei and A. esculentus*, which are the two common *Abelmoschus* species in Southern Nigeria and West Africa.

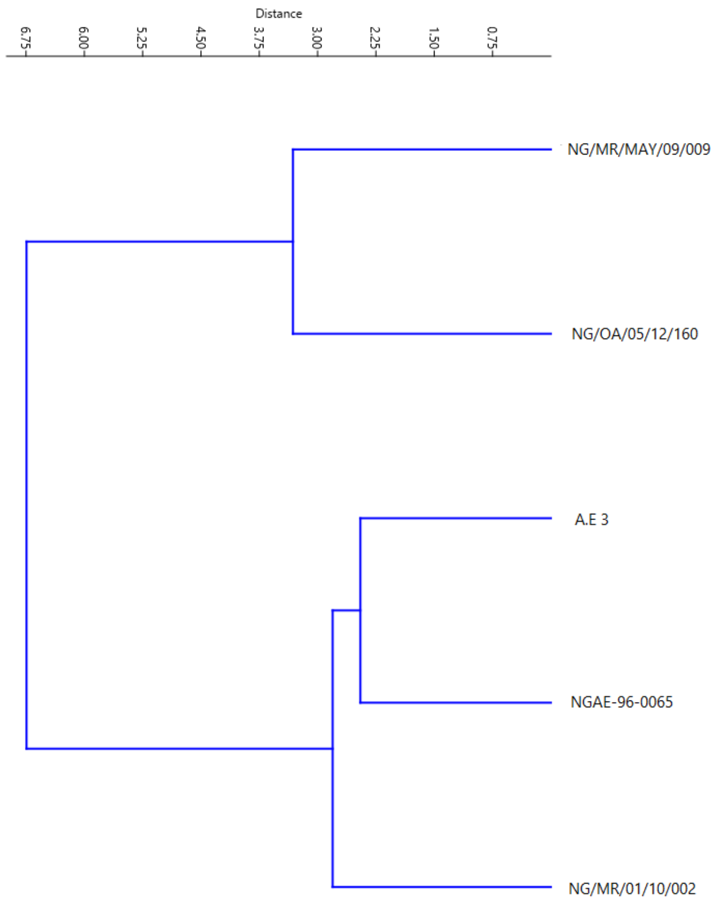

Figure 2: Dendrogram based on 16 qualitative morphological characters of five accessions of *Abelmoschus* species

Discussion

Variation is an important attribute in breeding programmes (Hazra and Basu, 2000; Omonhinmin and Osawaru, 2005). *Abelmoschus* species characterized in this study showed variation for eight traits including general aspect of the stem, nature of branching, fruit pubescence, fruit shape, position of the fruit on the main stem, leaf shape, fruit colour, and fruit length at maturity. These characters may allow for the identification, distinction, breeding and conservation of okra accessions in Nigeria and all over the world. This supports the findings reported in Yildiz et al. (2015); Ogwu et al. (2016) that fruit, leaf, and stem morphologies are the primary characteristics exploited to distinguish the okra accessions. The variation in general aspects of the stem, branching, leaf shape, petal colour, fruit colour, fruit shape, position of fruit on the main stem, fruit pubescence and length of peduncle are easily recognizable with visual approach by the use of colour chart and IBPGR, 1991 descriptor list of *Abelmoschus* species.

The observed variations in the growth habit and other characters may have evolved over time to suit environmental conditions. For instance, the erect stems allow for maximum and uniform exposure or distribution of all leaves and other vegetative parts for better interception of sunlight, and would also result in an increase in dry matter production and subsequent increase in yield (Oppong-sekyere et al., 2011). The intermediate nature of *Abelmoschus* species allows for larger and continuous fruit harvest. This is an advantage when the price of vegetables fluctuate (Oppong-sekyere et al., 2011). Variations in fruit characteristics may be indicative of differences in the genetic make-up of the plant (Ogwu et al., 2016). In addition, *Abelmoschus* species with determinate growth pattern and pronounced branching is likely *A. caillei* while those with indeterminate growth pattern and orthotropic branching as *A. esculentus* (Omonhinmin & Osawaru, 2005).

Moreover, Omonhinmin and Osawaru, (2005) reported that profuse branching in *Abelmoschus* may indicate high yield potential, as branches are production sites and hence the higher their number the greater the potential for yield. Profuse branching that was observed in NG/MR/01/10/002, A.E 3 and NGAE-96-0065 may be incorporated into NG/MR/MAY/09/009 and NG/OA/05/12/160 in order to produce *Abelmoschus* species that are less lodging with higher yield potentials for commercial production. Fruit with characteristics such as smooth, spineless, slender with green skin are desirable characteristics in Nigeria markets. Accession NG/MR/MAY/09/009 and NG/OA/05/12/160 display such characters. These can, therefore, be selected for breeding by crossing them with accession

NG/MR/01/10/002, A.E 3 and NGAE-96-0065, which are high yielding with longer harvest duration.

Results obtained in this study revealed variation in fruit colour among the five accessions. Accession NG/MR/01/10/002, NGAE-96-0065 and NG/MR/MAY/09/009 were green in colour while accession A.E 3 and NG/OA/05/12/160 were green with red patches. This was in accordance with the result of Oppong-sekyere et al., (2011). The results also suggest that the five accessions of *Abelmoschus* species exhibited varying degree of fruit pubescence including downy, slightly rough and prickly. Accessions NG/MR/MAY/09/009 and NG/OA/05/12/160 had downy fruit pubescence while accessions NG/MR/01/10/002 and NGAE-96-0065 had slightly rough fruit pubescence and accession A.E 3 showed prickly fruit pubescence. This was in accordance with the result of Thomas, (1991) and Bish et al., (1995). These features serve protective functions for the fruit.

The position of fruit on the main stem was pendulous in NG/MR/01/10/002, A.E 3 and NGAE-96-0065 and erect in NG/MR/MAY/09/009 and NG/OA/05/12/160. Omonhinmin and Osawaru (2005) reported that *Abelmoschus* spp with the pendulous position of fruit in relation to the stem are *A*. caillei while that one with erect to horizontal position was reported as *A. esculentus*. Several researchers Bish et al. (1995); Akinyele and Oseikita (2006); Oppong-sekyere et al. (2011); Omonhinmin and Osawaru (2005); Osawaru *et al*. (2013); Osawaru et al. (2014); Osawaru et al. (2015b); Osawaru *et al*. (2016) have investigated the diversity of different morphological traits within *Abelmoschus* species. Put together, their results suggest that a large number of *Abelmoschus* characters such as pigment colour and spines on fruit surface are inheritable suggesting that they are controlled by relatively few genes.

The multivariate analysis applied in this study was useful in tracing the possible relationship between the five accessions. Using multivariate techniques Osawaru et al., (2012) and other researchers showed its importance in numerical taxonomy. Twenty-one morphological characters were standardized, analyzed and subjected to principal component analysis and single linkage clusters analysis. The PC loading of each character on the different principal component axes is shown in Table 4 and was used to assess their relative contribution in showing variation. PC 1 was affected by characters such as general aspect of stem, branching, fruit pubescence, fruit shape, position of fruit on main stem and leaf shape. PC 2 was significantly loaded with characters such as fruit colour and fruit length at maturity. These characters account for the high variability observed in the accessions studied. However, principal component analysis alone would not give an adequate character representation in terms of relative importance when numerous characters are considered simultaneously (Shalini et al., 2003). To complement such result; scatter diagram was employed with idea of tracing the grouping tendency within the genus *Abelmoschus* as used by Wickremasinghe and Herat, (2006) and single linkage cluster analysis was also employed to classify the variation and show relationship pattern among the five accessions as used by Ariyo and Odulaja (1991). The scatter plots of PC 2 versus PC 1 (Figure 1) indicates a good tendency of grouping of the five accessions. Accession A.E 3 was grouped with NGAE-96-0065, this shows that the above accessions are to a certain extent, related morphologically. The five accessions were grouped into two distinct cluster groups with accession NG/MR/01/10/002, A.E 3 and NGAE-96-0065 all clustering in one and NG/MR/MAY/09/009 and NG/OA/05/12/160 160 (Figure 2). This could be used to identify them as having a common ancestral origin with a common gene. These variations within the genus *Abelmoschus* could be used for breeding that incorporates required traits and for proper classification. Hence, we recommend crosses to be done between accessions in the different cluster groups. From a phylogenetic perspective, all the accessions are likely descendants of the commonly cultivated *Abelmoschus* in Southern Nigeria and parts of West Africa.

Conclusion

This study highlighted the relevance of key descriptive *Abelmoschus* characters including general aspect of stem, branching, fruit pubescence, fruit shape, position of fruit on main stem, leaf shape, fruit colour and fruit length at maturity. These characters may be used to distinguish different *Abelmoschus* accession and can be exploited in the selection of okra germplasm for cultivation. The 16 morphological characters suggest that the five accessions probably belong to two *Abelmoschus* species. However, further studies using molecular characterization is required to confirm the findings reported in this study.

Acknowledgement

The authors appreciate the help of the National Center for Genetic Resources and Biotechnology, Ibadan, Nigeria.

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