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Evaluation of Nutritional Composition and Chlorophyll Content of Okra Accessions

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ABSTRACT

Okra (Abelmoschus esculentus [L.] Moench) is an important vegetable cultivated for the edibility of the fruit and the leaves as sources of vitamins, carbohydrate and protein in nearly all developing countries. In view of its importance, the genetic variability among some accessions of the crop was assessed for the improvement of its qualitative and quantitative traits. A total of twenty (20) accessions were obtained from National Centre for Genetic Resources and Biotechnology (NACGRAB), Ibadan. The accessions were evaluated for agro-morphological and yield parameters using a randomised complete block design (RCBD) with three replicates each. The nutritional and chlorophyll content were carried out under laboratory condition. Significant ($p < 0.05$) variation was observed in most of the parameters studied. The proximate compositions revealed that accession NGB00396 had the highest moisture content (18.22%), oil content (21.23%) and crude fibre (25.40%) but least in Nitrogen free extract (7.76%). Accessions NGB00469 and NGB00298 produced highest value in crude protein and nitrogen free extract contents with the value of 32.38% and 41.30% respectively. Highest chlorophyll a (22.72 mg/g) and b (18.49 mg/g) content was observed in NGB00331. Therefore, the high variability observed among the accessions for all the parameters studied indicate high genetic diversity in the crop. These variations however might be due to difference in their genetic makeup and environmental factors which can be exploited in further studies, notably, molecular characterization to ascertain the genetic bases in them and to arrange these accessions into suitable groups.

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INTRODUCTION

The flowering plant known as okra (*Abelmoschus esculentus* L.) is well-known for its culinary applications and nutritional value. This annual herbaceous plant, which belongs to the Malvaceae family (Sreenivas, 2024), is grown all over the world in tropical and subtropical areas (Rawat *et al.,* 2020) for its delicate, lengthy pods (Nwangburuka *et al.,* 2012). Many plant species, particularly vegetables, are widely recognized for their therapeutic qualities in a variety of international cuisines. While some are typically eaten raw, others are frequently grilled or fried. Okra (*Abelmoschus esculentus* L.) is a member of the Malvaceae family. Okra, which has its origins in Ethiopia, is popular throughout India under the names Bhindi (Hindi), Dhenras (Bengali), Bhindo (Gujarati), Vendai (Tamil), Bendekayi (Kannada and Telugu), Ventaykka (Malayalam), and Asra-pattraka (Sanskrit) (Sreenivas, 2024). It is one of the most significant fruits and vegetables grown and eaten all year round, particularly in Nigeria (Pathak et al., 2013). The leaves, buds, blooms, pods, stalks, and seeds of okra are rich in nutritional and therapeutic value, making it a very beneficial crop (Sreenivas, 2024). Because okra leaves and fruits create mucilaginous compounds, eating bulky foods like "eba," "pounded yam," and "fufu" is made easier in the majority of African cuisines (Nwangburuka *et al.,* 2012).

In addition to being a useful vegetable crop, okra's green pods are nutritious because they contain vitamins A, B, C, and K, folic acid, potassium, magnesium, calcium, and trace elements like copper, manganese, iron, zinc, nickel, and iodine (Yusif *et al.,* 2024), which are frequently absent from the diets of people in the

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majority of developing nations. As stated by Okanya *et al.* (2021). When compared to other plant protein sources, "okra" is a seed that is known to offer high-quality protein, especially when it comes to essential amino acid levels. It is more of a diet food than a staple. The young green pod is therefore a nutrient-dense vegetable with 86.1% moisture, 9.7% carbs, 2.2% protein, 0.2% fat, 1.0% fiber, and 0.8% ash (Yusif *et al.,* 2024). Mature okra seeds contain around 20% protein, and their amino acid composition is similar to that of soybean seeds. Furthermore, 20% of the seeds contain oil with a fatty acid concentration similar to that of cotton seed oil (Salama *et al.,* 2023).

The growing human population leads to a rise in the demand for food crops. Since it is generally recognized that stress conditions alter photosynthetic activity, which is strongly related to yield, many food producers continuously check the crop's health in order to acquire a higher producing crop (Pérez-Patricio *et al.,* 2018). Chlorophyll fluorescence, chlorophyll, calcium, and nitrogen levels are factors associated with crop health, according to Muñoz-Huerta *et al.* (2013). Nonetheless, chlorophyll content is frequently employed and has produced leaves that are adequate. This is because there is a strong link between plant health and chlorophyll concentration (Pérez-Patricio *et al.,* 2018).

Despite Okra's many nutritional advantages, there hasn't been much research done on its genetic improvement in worldwide research programs (Sanjeet *et al.,* 2010). This is mostly because Okra variations have a limited genetic base (Das *et al.,* 2012). Additionally, a thorough and concurrent investigation has been conducted to demonstrate the relationship between nutritional composition, yield, and the impact of leaf chlorophyll content on yield.

This study investigates the botanical traits, ascertain the content of chlorophyll a and b, and assess the nutritional compositions linked to various Okra accessions. This will contribute to a thorough understanding of Okra's multifaceted significance, which will in turn spark interest in improving Okra crops and their applications outside of their traditional locales.

MATERIALS AND METHODS

Collection of Materials

Twenty accessions of dry Okra (*Abelmoschus esculentus*) seeds were obtained from the Gene Repository Department of the National Centre for Genetic Resources and Biotechnology (NACGRAB) in Ibadan Oyo state, Nigeria. The accessions were labeled NGB00298, NGB00302, NGB00303, NGB00304, NGB00310, NGB00323, NGB00324, NGB00326, NGB00328, NGB00331, NGB00342, NGB00349, NGB00371, NGB00394, NGB00396, NGB00397, NGB00452, NGB00466, NGB00467 and NGB00469.

Experimental Site

The field and laboratory studies were conducted in the Plant Biology Department's Experimental Garden and Laboratory at the Federal University of Technology in Minna, Niger State. The Southern Guinea Savannah region of Nigeria is home to Niger State. The city of Minna is located in Nigeria's North Central area in latitude 9^{o} 37' North and longitude 6^{o} 33' East. Ukubuiwe *et al.* (2016) state that there are two primary seasons in the climate: a wet season that runs from May to October and a dry season that runs from November to April. The average temperature is between 22 and 33.3 degrees Celsius, and the average annual precipitation is between 1200 and 1300 mm.

Experimental Design (Field studies)

The study consists of three Randomized Complete Block Design (RCBD) replicates. The trial land area was cleared of grass using a cutlass before being plowed and ridged with a hoe. The seeds of two healthy accessions were planted two centimeters deep. Three weeks after sowing, the immature okra seedlings were pruned to ensure the proper population. To encourage rapid growth, the seedlings were lightly hydrated every morning and evening for a week using a watering can. There were a total of 60 plant stands, and each following crest was separated by a constant 60 cm. N.P.K. Fertilizer was sprayed at 15:15:15 three weeks after planting and again throughout

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flowering. Physical weeding was done three, six, and nine weeks after planting.

Collection of Data

Proximate composition: Using the AOAC (2003) methodology, the proximate composition of okra fruits was determined to ascertain the percentage of moisture, crude protein, crude fiber, crude fat, ash, and nitrogen free extract (carbohydrate) in order to assess its nutritional value in human diets.

Determination of moisture: The oven drying method was used to determine the moisture content. A clean, dry crucible was used to precisely weigh 1.5 g of thoroughly mixed sample (W1). For six to twelve hours, the crucible was kept in an oven at 100 to 105 degrees Celsius until it reached a consistent weight. The crucible was then allowed to cool for half an hour in the desiccator. It was weighed once again after cooling (W2). The following formula was used to get the percentage moisture content:

$$\% \text{ moisture} = \frac{w1 - w2 \times 100}{\text{wt.of sample}}$$

Where W_1 = Initial weight of crucible + Sample, W_2 = Final weight of crucible + Sample

Determination of crude protein: According to Pearson (1976), the nitrogen value, a precursor of protein, was used in the Kjeldahl method to determine the amount of protein in the sample. In order to digest the samples, concentrated sulfuric acid (H_2SO_4) was heated while the digestion mixture was present. After that, the mixture was turned alkaline. Ammonium sulfate thus produced, released ammonia which was collected in 2% boric acid solution and titrated against standard HCl. The amount of nitrogen was multiplied by the required factor (6.25) to determine the total protein, which was then computed as

% Crude Protein = 6.25* x %N (*. Correction factor)

$$\%N = \frac{TV \times 0.014 \times MA \times df \times 100}{\text{wt.of Sample}}$$

Where TV = Titre value, 0.014 = Nitrogen standard, N = Normality of HCl, MA = molarity of acid, df = dilution factor after digestion

Determination of crude fiber: Diluted H2SO4 and then diluted KOH solution were used to digest a moisture-free, ether-extracted sample of cellulose-based crude fiber. Crude fiber was measured as the weight loss following ignition of the undigested residue that was collected after digestion. The following formula is used to determine crude fiber:

$$\% \text{ Crude fibre} = \frac{A - B \times 100}{C}$$

Where A = weight of dry sample, B = weight of ashed sample, C = weight of sample boiled.

Determination of ash: To determine the amount of ash, a clean, empty crucible was heated to 600 degrees Celsius for an hour in a muffle furnace, cooled in a desiccator, and its weight was recorded (W1). Each sample weighed one gram and was placed in crucible (W2). Using a blowpipe, the sample was ignited over a burner until it was blackened. The crucible was then heated to 550 degrees Celsius for two to four hours in a muffle furnace. All of the organic stuff in the sample has completely oxidized, as seen by the formation of gray-white ash. The furnace was turned off after ashing. After cooling, the crucible was weighed (W3). The following calculation was used to determine the percentage of ash:

$$\% \text{ Ash} = \frac{\text{Difference in wt.of Ash}}{\text{wt of Sample}} \times 100$$

Where Difference in wt. of Ash = W_3 -W_1

Determination of crude fat: Fat was measured using an intermittent Soxhlet extraction device using the dry extraction (ether extract) method. Filter paper was used to wrap about 1 g of the moisture-free sample, which was then put in a fat-free thimble and put into the extraction tube. After being weighed, cleaned, and dried, the receiving beaker was filled with petroleum ether and placed within the device. To begin extraction, turn on the heater and water. Before the final siphoning, let the ether evaporate after 4-6 siphonings and unplug the beaker. After ether

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washing and evaporating the ether in a water bath, the extract was transferred onto a clean glass dish. The dish was then chilled in a desiccator after being baked for two hours at 105 degrees Celsius. The following formula was used to calculate the percentage of crude fat:

$$\% \text{ Crude Fat} = \frac{\text{wt.of ether extract x 100}}{\text{wt.of Sample}}$$

Determination of nitrogen free extract: Nitrogen Free Extract (NFE) was computed using differences following the proximate analysis of all other components.

NFE = (100 - % moisture + % crude protein + % crude fat + % crude fiber + % ash)

Statistical Analysis

Using SPSS software version 21, data for the study were gathered directly from the field and laboratory in each okra accession in accordance with standard procedures. They were then combined for statistical Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT) to separate means where needed. A significant difference was defined at $P < 0.05$.

Chlorophyll analysis: The Arnon (1949) method was used to estimate the amount of chlorophyll a and chlorophyll b. Each Okra accession's fresh leaf samples were gathered, cleaned with distilled water, and then readyed for chlorophyll analysis. A spectrophotometer was used to measure the resulting color intensity at 665 and 645 nm. The following formulas were used to estimate photosynthetic pigments using the acquired values, which were represented in mg/g fresh weight of leaves:

Chlorophyll a (mg/g) = [11.6 (OD 665) – 1.3(OD 645)]

Chlorophyll b (mg/g) = [19.1 (OD 645) – 4.7 (OD 665)]

Where OD 665 and OD 645 are the absorbance measured from 665 nm and 645 nm respectively.

RESULTS

Proximate Compositions of Okra Accessions

Moisture content

The moisture content of the okra accessions varied significantly ($p < 0.05$). But the accession with the highest moisture content, NGB00396 (18.22%), had the lowest moisture content, NGB00467 (7.57%), which was substantially different ($p < 0.05$) from all the other accessions but considerably the same as NGB00302 (7.75%) (Table 1). The fruit's varying ages at harvest may be the cause of this outcome.

Ash content

NGB00452 had the least ash content (4.29 %) which was significantly different ($p < 0.05$) from all other accessions. The highest ash content (9.83 %) was however observed in NGB00469 which was significantly the same ($p > 0.05$) with accessions NGB00298, NGB00302, NGB00303, NGB00304, NGB00323, NGB00324, NGB00326, NGB00328, NGB00342, NGB00349, NGB00371, NGB00396, NGB00397, and NGB00467 (Table 1).

Oil content

The highest oil content was observed in NGB00396 (21.23 %) which was significantly the same ($p < 0.05$) with the oil content of NGB00469 (19.56 %). The least oil content was observed in NGB00371 (6.08 %) which was significantly different ($p < 0.05$) from other accessions (Table 1). This result however could be attributed to the number of seeds in the fruit. Thus, the Okra accessions can be grouped into low, moderate and high oil content types.

Crude fibre

The highest crude fibre (25.40 %) was recorded in NGB00396 and the lowest (8.19 %) was observed in accession NGB00326. Values of crude fibre among the accessions differ significantly ($p < 0.05$) (Table 1). This difference in fibre content could be attributed to the age and size of the fruit at harvest as it is relatively possible for aged fruit to contain more fibre.

Crude protein

Significant differences ($p < 0.05$) were observed in the crude protein values of all the accession. The highest crude protein was observed in NGB00469 (32.38 %) which was

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significantly different ($p < 0.05$) from all other accession while the lowest crude protein value was observed in NGB00304 (16.63 %) followed by NGB00298 (17.50 %) and NGB00371 (17.51 %). There is however moderate significant difference among the other accessions (Table 1).

Nitrogen free extract

The results of the Nitrogen Free Extract shown in Table 1 reveals the Okra accessions significantly differs ($p < 0.05$) from the other in the level of Nitrogen Free Extract (NFE). Accession NGB00396 produces the least (7.76 %) NFE while accession NGB00371 recorded the highest (44.78 %). This value indicates that there is great significant difference ($p<0.05$) among the Okra accession which could be attributed to difference in their energy storage potential through photosynthesis.

Table 1: Proximate Analysis of Fruits of Okra Accessions

Accessions	MC (%)	AC (%)	OC (%)	CF (%)	CP (%)	NFE (%)
NGB00298	10.18±1.03^{bc}	7.69±0.19^{c}	8.95±0.92^{bc}	12.99±1.15^{bc}	17.50±1.01^{ab}	41.30±0.78^{i}
NGB00302	7.75±0.32^{a}	7.36±0.23^{bc}	10.24±1.04^{cd}	18.49±0.74^{de}	21.97±1.06^{de}	34.44±0.31^{gh}
NGB00303	16.16±0.31^{jk}	7.14±0.22^{bc}	17.22±0.18^{i}	15.24±0.24^{c}	23.63±0.53^{efg}	19.29±.22^{d}
NGB00304	15.31±0.79^{ij}	7.48±0.89^{c}	14.25±0.89^{fgh}	17.51±0.35^{d}	16.63±0.60^{a}	28.70±0.91^{f}
NGB00310	10.89±0.53^{bcde}	8.45±0.03^{d}	13.60±1.02^{fg}	11.75±0.28^{b}	23.63±0.21^{efg}	30.82±0.14^{g}
NGB00323	17.23±1.03^{kl}	7.87±0.19^{c}	17.17±0.39^{i}	17.76±0.48^{d}	25.38±.79^{g}	14.62±0.76^{c}
NGB00324	12.40±0.44^{efg}	7.88±0.62^{c}	10.03±0.64^{cd}	11.81±0.16^{b}	19.79±0.61^{bcd}	37.03±1.15^{h}
NGB00326	13.31±0.39^{fgh}	7.53±0.88^{c}	14.71±0.69^{fgh}	8.19±0.41^{a}	19.25±0.42^{bc}	37.09±0.22^{h}
NGB00328	14.31±0.37^{hi}	7.28±0.95^{cd}	11.31±0.57^{de}	18.43±0.69^{de}	23.84±1.66^{efg}	25.97±0.66^{e}
NGB00331	11.88±0.74^{cdef}	9.40±0.36^{ef}	16.18±0.82^{hi}	17.03±0.65^{d}	25.15±1.19^{fg}	20.15±0.10^{d}
NGB00342	9.92±0.11^{b}	7.54±0.81^{c}	10.76±0.64^{cd}	10.70±01.05^{b}	21.98±0.67^{de}	39.13±0.79^{hi}
NGB00349	14.00±0.72^{ghi}	7.93±0.42^{c}	13.64±0.67^{fg}	15.79±1.00^{c}	25.38±0.79^{g}	24.81±0.23^{e}
NGB00371	10.56±0.36^{bcd}	7.56±0.25^{c}	6.08±0.71^{a}	13.35±.93^{bc}	17.51±1.00^{ab}	44.78±.87^{j}
NGB00394	10.44±0.84^{bcd}	6.48±0.39^{b}	7.77±0.47^{ab}	19.74±0.78^{e}	20.28±0.22^{cd}	35.55±0.90^{h}
NGB00396	18.22±0.39^{l}	7.38±0.34^{bc}	21.23±0.49^{j}	25.40±0.65^{f}	20.19±0.45^{cd}	7.76±0.48^{a}
NGB00397	12.16±0.12^{def}	7.56±0.42^{c}	13.31±0.7^{1efg}	13.93±0.07^{bc}	22.75±0.42^{ef}	30.27±0.42^{g}
NGB00452	9.66±0.35^{b}	4.29±0.22^{a}	15.34±0.41^{ghi}	19.28±0.37^{e}	20.07±0.55^{cd}	31.67±0.82^{g}
NGB00466	14.97±0.05^{hij}	9.17±1.08^{de}	17.06±0.71^{i}	18.96±0.85^{de}	20.13±0.69^{cd}	19.72±0.74^{d}
NGB00467	7.57±0.33^{a}	7.54±0.81^{c}	12.94±0.92^{ef}	18.73±0.79^{de}	20.04±0.04^{cd}	33.30±0.53^{gh}
NGB00469	14.26±0.48^{hi}	9.83±0.09^{f}	19.56±0.86^{j}	14.06±0.08^{bc}	32.38±0.36^{h}	9.80±0.49^{b}

Values are Mean ± Standard Error of mean. Values followed by different subscript(s) along the column are significantly different at $p < 0.05$.

MC = Moisture Content, AC = Ash Content, OC = Oil Content, CF = Crude Fiber, CP = Crude Protein, NFE = Nitrogen Free Extract

Chlorophyll Contents of Okra Accessions

The accession with highest chlorophyll a content was observed in NGB00331 (22.72 mg/g). This was significantly different ($p < 0.05$) from Chlorophyll a content of all other accession while NGB00397 had the lowest Chlorophyll a content (7.79 mg/g) which was significantly the same ($p > 0.05$) with accession NGB00304 (8.01 mg/g) and NGB00342 (9.54 mg/g) but significantly different ($p < 0.05$) from other accession (Table 2). Similarly, NGB00331 recorded the highest chlorophyll b content (18.49 mg/g). The result was significantly different ($p < 0.05$) from all the other accession whereas the lowest chlorophyll b content (6.49 mg/g) was observed in accession NGB00349 which was significantly the same ($p > 0.05$) with NGB00302, NGB00304, NGB00323, NGB00324, NGB00342, NGB00397 and NGB00469 but significantly different ($p < 0.05$) from the other accessions (Table 2). These result could be attributed to the number of branches, in that, it is an indication for more

leave per plant which consequently result in chlor ophyll content.

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Table 2: Chlorophyll Contents and Okra Accessions

Accessions	Chlorophyll A (mg/g)	Chlorophyll B (mg/g)
NGB00298	16.53±0.63^{g}	9.47±0.96^{bcde}
NGB00302	15.13±0.09^{efg}	7.96±0.65^{abc}
NGB00303	12.84±0.18^{bcd}	9.62±0.36^{bcde}
NGB00304	8.01±0.83^{a}	8.38±0.43^{abc}
NGB00310	12.93±0.75^{bcd}	8.56±0.52^{bc}
NGB00323	11.74±0.73^{b}	8.33±0.38^{abc}
NGB00324	14.67±0.19^{defg}	8.00±1.16^{abc}
NGB00326	20.95±1.37^{ij}	13.87±0.07^{f}
NGB00328	20.18±0.65^{hi}	13.97±0.36^{f}
NGB00331	22.72±1.57^{j}	18.49±0.30^{g}
NGB00342	9.54±0.31^{a}	7.71±0.86^{ab}
NGB00349	12.39±0.90^{bc}	6.49±0.55^{a}
NGB00371	15.32±0.18^{efg}	9.77±0.20^{cde}
NGB00394	18.46±0.38^{h}	11.05±1.13^{e}
NGB00396	16.34±0.46^{fg}	9.96±0.85^{bcde}
NGB00397	7.79±0.39^{a}	8.03±0.49^{abc}
NGB00452	14.21±0.11^{cdef}	8.75±0.27^{bcd}
NGB00466	20.58±0.49^{i}	13.69±0.33^{f}
NGB00467	16.31±0.44^{fg}	10.62±0.29^{de}
NGB00469	13.61±0.35^{bcde}	8.24±0.66^{abc}

Values are Mean ± Standard Error of mean. Values followed by different subscript(s) along the column are significantly different at $p < 0.05$

DISCUSSION

The proximate analysis revealed percentage differences in the okra accession fruits' nutritional makeup. The fruit of NGB00469 has the highest protein content (32.38%). According to Varmudy (2011) and Falusi *et al.* (2012), this protein content is consistent with the protein content in pods. All of the okra accessions under investigation had comparatively high fat contents. This is obviously in contrast to Nwachukwu *et al.* (2014), who found that okra has a low-fat content. However, Adetuyi *et al.* (2011) revealed that the oil content ranges from 9.22 to 10.57% per 100 g fresh state. NGB00371 had the highest carbohydrate content of 44.78 %, whereas NGB00396 had the lowest fat content of 7.76%) These might be explained by variations in their capacity for photosynthesis-based energy storage. The outcome is consistent with Benchasri's (2012) finding that okra pods had an 8.20% carbohydrate content.

Any plant's leaves are where photosynthetic processes take place. One could assume that a low or high photosynthetic capacity is the cause of an increase or decrease in crop yield. According to Ahiakpa *et al.* (2013), a higher leaf area index and consequently a higher fraction of intercepted radiation and its usage efficiency contribute to high photosynthetic capacity. On the other side, a reduction in their photosynthetic capacity could have detrimental effects on the crop's ability to produce assimilate. Among other factors, the higher chlorophyll a and chlorophyll b content of NGB00331 may have allowed this accession to create more assimilates during their photosynthetic activities, which is why this accession performed better in this study in terms of the number of seeds per fruit.

CONCLUSION

The okra accessions employed in this study have been shown to exhibit a significant amount of variety, as well as the discovery of novel nutritional characteristics and superior yield

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attributes. Because of its ideal fat and ash levels, as well as its greatest protein content (32.38%) and comparatively low carbohydrate content (9.80%), NGB00469 was found to be a very good addition. With a high producing accession, it might be chosen as an elite parent accession for use in improvement projects. However, NGB00371 and NGB00396 with high oil content had the largest energy (carbohydrate) content (44.78%). A high level of chlorophyll a and b was found in Accession NGB00331. This suggests a strong photosynthetic potential in relation to the amount and quality of crop yield. Nigerian okra improvement initiatives can benefit from the diversity found among all the okra accessions included in this study. Techniques like hybridization might also be employed to produce a variation that might share the beneficial characteristics. As it has been thoroughly investigated, it is thought that this information would be very helpful to breeders and agronomists in their development programs in Nigeria and elsewhere. This would help farmers grow better varieties and increase their revenue.

RECOMMENDATION

In order to determine the close and distant relationships between the various accessions, a more thorough investigation is advised, particularly with regard to the molecular characterization of the crop. Also, to verify the persistence of the favorable features reported in the current study, the accessions with superior qualities should be subsequently assessed.

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