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Preliminary Studies on Fruit Lignification Time Interval and Phenological Traits of Selected Okra (*Abelmoschus esculentus* L Moench) Genotypes Grown in Southern Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. Author GMU designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors ISE, MBO, UW and JOJ managed the literature searches. Authors GMU and UW performed the analysis. Author GMU managed the experimental process and developed the protocol and method for time interval measurement. All authors read and approved the final manuscript.

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ABSTRACT

Aims: To determine the time interval between fruit formation and lignification (hardening of fruits) and phonological traits in three short, early maturing and three tall, late maturing genotypes of Okra (*Abelmoschus esculentus* L Moench) grown in southern Nigeria.
Study Design: Two separate experiments for the dwarf early maturing and tall late maturing genotypes were laid out in a randomized complete block design with five replications.

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Place and Duration of Study: The study was conducted in Calabar rainforest agro-ecology in 2014 and 2015 vegetables growing seasons.
Methodology: Fruit lignification time interval was determine using the direct time measurement using stop watch and observation of the time interval between fruit formation to time of hardening (Lignification). Data for phenological traits were generated from direct measurements using appropriate tools and observations of okra plants in the field.
Results: Results showed that the tall, late maturing okra genotypes required longer time interval to become lignified compared to the dwarf, early maturing genotypes throughout the period of study. Results of time interval measurement showed that significant ($p<0.05$) differences were detected among genotypes. A time interval of 142±4.50 hours was required for fruit lignification in Perkins long pod, while 'Etighi idok' and 'Okpo-mbontam' genotypes required 124±3.40 and 96±5.15 hours respectively to become lignified. The dwarf, early maturing genotypes, NHAe-47-4 fruits become lignified after 124±3.45 hours, 'Asaka awum' fruits required 121±4.20 hours while Agwu early took 78±3.25 hours to become unusable in fresh conditions. Results of phenological traits of Okra genotypes also revealed that the tall, late maturing genotypes had more branches per plant, more fruits per plant, longer fruiting period and longer days to flower and fruiting initiation compared to the dwarf, early maturing genotypes.
Conclusion: Knowledge of fruit lignification time interval in Okra will no doubt reduce the laborious task of daily handpicking of immature fruits, reduce the rate of spoilage of the vegetable, enable for the consumption of fresh rather than dried okra and for plan and controlled harvesting of fruits for the market, income and food security.

Keywords: Fruit lignification; phenological traits; okra genotypes; time interval; Abelmoschus esculentus.

1. INTRODUCTION

Okra, also known locally as 'Gombo', 'Ntonghi', 'Etighi' or lady's finger, is one of the crop plant used as vegetable for the longest time throughout history. The Ethiopians and Egyptians used it some millennium years ago [1]. Today, Okra is grown primarily in the Tropical regions of Africa, Asia and America.

The fruit of okra is an annual herbaceous plant of the botanical family Malvaceae. It reaches a height of 1 – 5 m depending of cultivar. The fruits are greenish in colour and covered with fine hair-like structure. The fruit has an elongated shape and its size ranges between 3 – 20 cm.

Okra is originally from Ethiopia from where its cultivation has extended throughout the hotter regions of the entire world. It is highly valued in the middle-east, India, East Africa, Nigeria, Thailand and the United states.

Okra is notable for its protein content which is quite high for a vegetable. In addition to its richness in vitamins and minerals, Okra fruits have remained a household recipe for the preparation of vegetable soup and other delicacies due to its high content of nutritious soluble mucilage which is locally believed to boost semen production in humans [2]. Okra is also rich in soluble mucilage fibre which exercises a protective and emollient function within the digestive tract [3].

Okra fruits are known to initiate lignification and become hardened soon after the fruits are formed and set. The time interval between fruit formation and fruit hardening through lignification vary among Okra genotypes and depends on available mineral nutrients, environmental factors, moisture, pests and diseases [1,2,3,4].

In most communities in Southern Nigeria, the lignified Okra fruits are processed into "Dried Okra" and preserved dried for future soup preparations. Although, this could be viewed as alternative way of utilizing Okra fruits that have been lignified, however, research have also shown that the mineral and nutritional composition and content of the fruit decreases with increase in lignin and fibre content of the fruits [5].

The dearth of information on fruit lignification time interval in Okra genotypes is an indication of the protracted decline in the cultivation and production of Okra by local farmers, thus making the large –scale production of the okra difficult.

Fruit lignification in Okra is a biochemical and physiological process which takes place inside

the fruits resulting to the conversion of simple and soluble carbohydrates in the fruits to complex and insoluble carbohydrate polysaccharide compound called lignin which is deposited as dry matter within the cellulose cell walls of Okra during secondary thickening, thus conferring characteristics such hardiness, woodiness, toughening of tissues and rigidity of fruits making them resistant to tear and impermeable to water and gases [5,6]. This biochemical and physiological attribute of fruit lignification calls for the daily hand picking of small fruits, which are yet to attain physiological maturity and fruit size, thus making Okra production laborious and uneconomical [6].

The biochemical and physiological processes leading to Fruit lignification has made research efforts in Okra fruits much more difficult and thus left Okra vegetable fruit production to the local initiative of the resource poor farmers, despite the nutritional importance of the crop in human and animal dietary needs [7].

Fruit lignification in okra has often led to the production of more dry okra fruits in the field, which the poor resource farmers have often preserved and used as foundation seeds in subsequent farming seasons. This has led not only to poor yield, but also in the production of genetically impure, low yielding and non-resistant cultivars due to inbreeding depression [5,8]. The attendant consequence of this phenomenon and losses to Okra farmers cannot be over-emphasized.

Fruit lignification in Okra has also defied conventional storage practices [9,10,11,12] for fresh fruits and hence remains a source of concern for breeders, agronomists, horticulturists, nutritionist and farmers alike.

This present study therefore seeks to generate the much needed knowledge by researchers and farmers alike on fruit lignification time interval for selected okra (*Abelmoschus esculentus*) genotypes to enable for adequate mechanization practices, planned harvesting of fresh fruits and large scale production of okra.

2. MATERIALS AND METHODS

2.1 Study Location

The study was carried out in Calabar in 2014 and 2015 early and late vegetable cropping seasons.

2.2 Source of Planting Materials

Three dwarf, early maturing Okra genotypes, NHAe -47-4; 'Asaka awum" and Agwu early and three tall, late maturing okra genotypes, 'Etighi Idok', Perkins long pod and 'Okpo Mbontam', were used for the study. Early maturing genotypes were obtained from specialized dealers in Calabar while the late maturing genotypes which were mostly local cultivars were obtained from local farmers.

2.3 Experimental Design and Layout

The experiment was set –up in the compound garden field measuring 30 m x 33 m. The field was divided into three blocks with five replicates. Each experimental unit or plot measured 4 m x 3 m. The field was laid out in a Randomized complete block design (RCBD).

The field experiments were divided into two. In experiment one, three dwarf, early maturing Okra genotypes were planted out 1 m apart in the plots at 2 seeds per hole, which was later thinned to a plant per stand to give a plant density of 12 per plot.

In experiment two, three tall late maturing Okra genotypes were planted 1 m apart as in experiment one. Routine agronomic and cultural practices associated with okra cultivation were adopted and maintained throughout the study. A blanket treatment of 200 g of cow-dung (organic manure) was applied per stand for all experimental plants in both experiments.

Fig. 1. Dried lignified Okra fruit use as seeds

Fig. 2. Fresh unlignified Okra fruits

Fig. 3. Dwarf early maturing Okra genotypes

Fig. 4. Tall late maturing Okra genotypes

All Figs. 1, 2, 3 and 4 above are as obtained from local farmers.

2.4 Experimental Model for Fruit Lignification Time Interval Determination

The basic Tenus and Michaelis-Mentens enzyme model can be modified and adopted for the determination of time interval for fruit lignification in Okra. However, this can only be achieved with some basic assumptions which are stated below

$$\frac{\delta\varepsilon}{\delta\tau} = a\mu + b\delta\mu + \frac{k\varepsilon}{\delta\varepsilon} \quad (1)$$

$$\delta\tau = a\mu + b\delta\mu + k\varepsilon \quad (2)$$
$$\tau = a\mu + b\mu + k\varepsilon$$

Assumptions here are that

a, b and k are model constants given in hours.

where a and b are greater or equal to 24 hours

ε = specific rate of dry matter or mineral accumulation in Okra fruit
μ = fruit specific growth rate
δ = change or differential
τ = fruit lignification time interval in hours

For this present study, the model was not adopted because of lack of equipment for determining rate of dry matter accumulation and growth rate of fruit.

2.5 Direct Timing and Measurement of Lignification Time Interval

This method was adopted for the measurement of time interval for fruit lignification for the different okra genotypes throughout the duration of the experiment as described below.

$$Fruit\ lignification\ time\ interval = Final\ time\ of\ fruit\ lignification - initial\ time\ of\ fruit\ formation$$

$$FLTI = FTFL - ITFF \quad (3)$$

2.6 Data Collection Procedures

The experiment was located and carried out in compound garden and backyard to facilitate easy access to test crops at odd hours of the night, observations and time measurement. Fruit lignification interval was obtained by recording the time interval between fruit formation (newly formed fruits) and fruit hardening per plant. Daily recording of time interval and number of new fruits formed and their time of hardening was

done for one month for all the treatments and genotypes.

Five fruits per plant were marked with black dye as they developed from flower buds in the centre of each plot of 2 m x 1 m, as sampling area. The time for each fruit formation was recorded as initial time for each fruit per plant. These were monitored day and night at 30 minutes interval using penetrometer to observe for lignification throughout the study. Lignified okra fruits were observed through rigidity, woodiness, hardiness and resistance of fruit to mechanical tear with high penetrometer readings and the time this happened were recorded as final time for lignified fruits.

Fruit lignification time interval was measured and estimated as the time differences between initial fruit formation time and the final time when fruits became lignified.

Data for phenological traits such as days to flower initiation, fruit width (cm), branches per plant, fruits per plant, plant height (m), fruiting period (days) and fruit length were collected at fortnight interval and at fruiting. Data generated were subjected to statistical analysis using Genstat Software Version 5 for analysis of variance (ANOVA) in a randomized complete block design (RCBD). Treatment means with significant differences were separated using the Fishers' least significant difference (LSD) test at 5 % probability level.

3. RESULTS

Results of phenological attributes of okra (*Abelmoschus esculentus*) fruits for the short and early maturing genotypes presented in Table 1 showed that mean flower initiation days differed ($p<0.05$) significantly among NHAe-47-4, 'Agwu early' and 'Asaka Awum' genotypes in 2014 and 2015. The NHAe-47-4 genotype required 26 days to initiate flowering on the average while the Agwu early and Asaka awum took a mean of 32 and 29 days to initiate flowering respectively. No significant ($p>0.05$) differences were detected for fruit length for the short maturing okra genotypes for the two years of study. However, fruits from NHAe-47-4 were longer while shorter fruits in were measured from 'Asaka awum' and 'Agwu early'. Similarly, fruit width did not differ ($P>0.05$) significantly among the early maturing genotypes. The early maturing genotypes were dwarf and short in all three genotypes showing no remarkable differences in plant heights. Number of branches per plant did not differ ($p>0.05$) among the early maturing genotypes.

Results for the tall late maturing okra genotypes presented in Table 1 revealed that flower initiation days for both seasons were significantly ($p<0.05$) different among the selected genotypes. Perkin long pod took shorter months, while Etighi idok and Okpo-mbontam required more months to initiate flowering. Similarly, fruit length showed significant ($p<0.05$) differences amongst genotypes evaluated for both seasons. Perkins long pods had longer fruits while Okpo-mbontam and Etighi idok had shorter fruits.

The results further revealed that the fruit lignification time interval (FLI) between fruit formation and fruit lignification differed ($p<0.05$) significantly among all the genotypes evaluated for the two cropping seasons.

Table 1. Means and standard errors of some phonological attributes for dwarf early maturing and tall late maturing okra (*Abelmoschus esculentus*) genotypes for 2014 and 2015

Okra genotypes early maturing	DFI	FL (cm)	FW (cm)	PH (m)	BPP	FPP	FI (days)
NHAe-47-4	26 ±4.0	6.47±0.04	3.91±0.50	1.61±0.14	3.00±0.20	24±3.0	22±2.0
'Asaka Awum'	29±6.0	5.21±0.20	3.66±0.80	0.87±0.07	2.00±0.10	21±2.0	18±4.0
'Agwu Early'	32±3.0	4.69±0.03	3.01±0.23	0.91±0.11	2.00±0.10	19±3.0	14±3.0
LSD	**1.12***	**NS**	**NS**	**NS**	**NS**	**1.23***	**2.11***

Late maturing	DFI	FL (cm)	FW (cm)	PH (m)	BPP	FPP	FI (days)
Perkins LP	98 ±10.0	12.18±1.04	2.98±0.50	3.74±0.04	16±2.0	116±4.0	89±5.0
'Etighi idok'	121±15.0	7.89±0.80	2.50±0.09	3.61±0.47	12±3.0	102±2.0	77±4.0
'Okpo-mbontam	132±12.0	6.11±0.13	2.61±0.22	3.59±0.89	14±1.0	99±3.0	69±3.0
LSD (0.05)	**4.09***	**2.01***	**NS**	**NS**	**1.14***	**2.12***	**5.33***

Data are means and standard errors of five (5) replications for 2014 and 2015 cropping seasons.
**= Significant at 5 percent level of probability, NS= Not significant at (p<0.05), DFI = Days to flower initiation, PF = Period of fruiting, FPP = Fruits per plant, PH = Plant height (m), BPP = Branches per plant, FL=Fruit length (cm), FW= Fruit width (cm), FI = Fruiting Interval (days)*

Results presented in Table 2 showed that the early maturing genotypes showed a shorter interval when compared to the late maturing genotypes in terms of fruit lignification interval. For the early maturing genotypes, results of 2014 showed that NHAe-47-4 fruits became lignified at interval of 114±3.50 hours after fruit formation. 'Asaka awum' fruits became lignified after 112±3.40 hours of fruit formation, while 'Agwu early' fruits became lignified after 79±3.20 hours of fruit formation.

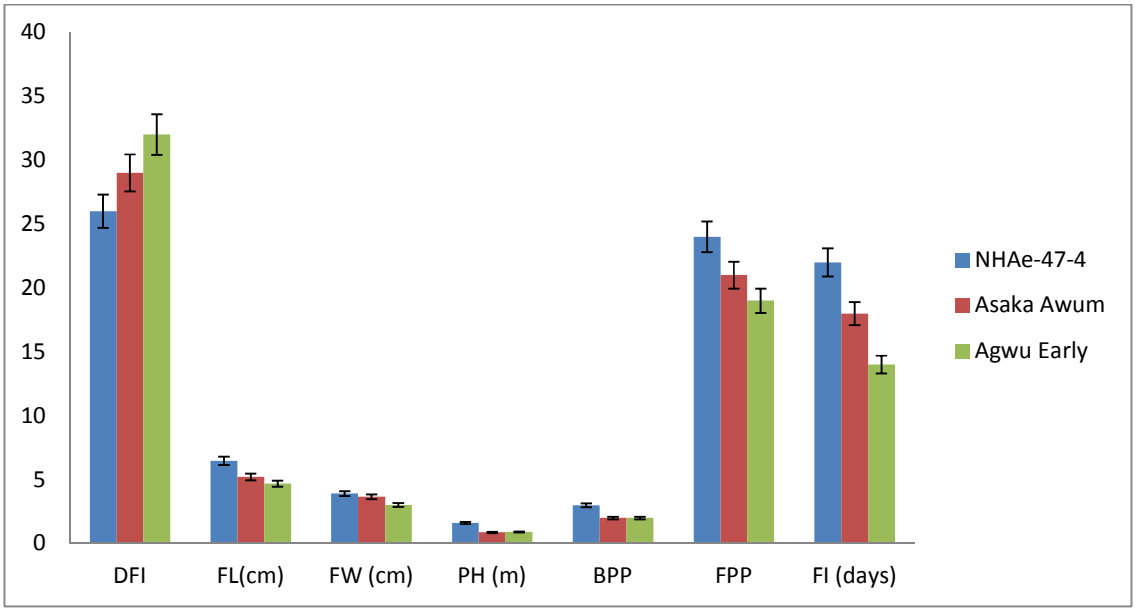

Fig. 5. Chart showing phenological traits of dwarf, early maturing okra gentypes

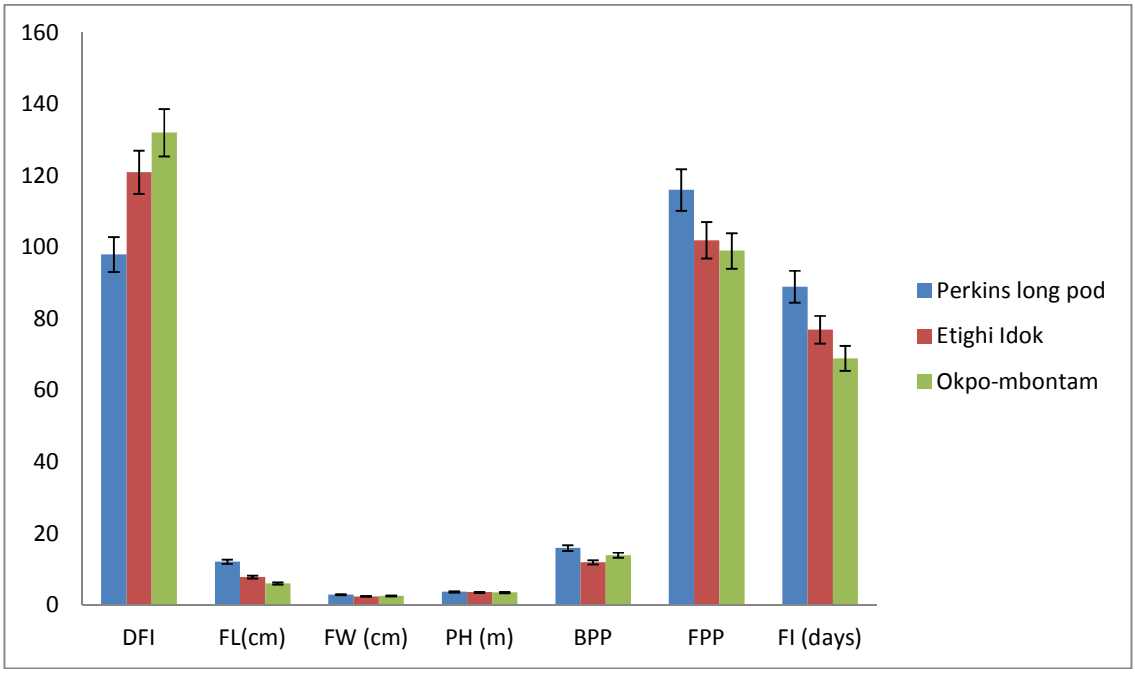

Fig. 6. Chart showing phenological traits of tall, late maturing okra genotypes

In 2015, NHAe-47-4 and 'Asaka awum' fruits became lignified after time intervals of 124±3.45 hours and 121±4.20 hours of fruit formation respectively, while it took a time interval of 78±3.25 hours for fruits of 'Agwu early' to become lignified after formation.

For the late maturing okra genotypes, results of 2014 showed that 'Etighi idok' and Perkins long pod fruits became lignified after 122±2.30 hours and 139±6.45 hours after fruit formation respectively. 'Okpo-mbontam' fruits became lignified after 90±4.40 hours of formation. Results of 2015 revealed that fruits of 'Etighi idok', Perkins long pod and 'Okpo-mbontam' became lignified after 124±3.40 hours, 142±4.50 hours and 96±5.15 hours respectively.

Table 2. Means and standard errors of fruit lignification time interval for dwarf early maturing and tall late maturing okra (*Abelmoschus esculentus*) genotypes fruit lignification time interval

Okra genotypes early maturing	2014 time (hours)	2015 time (hours)
NHAe-47-4	114±3.50	124±3.45
'Asaka Awum'	112±3.40	121±4.20
'Agwu Early'	79±3.22	78±3.25

Late maturing	2014 time (hours)	2015 time (hours)
Perkins Long Pod	139±6.45	142±4.50
'Etighi idok'	122±2.30	124±3.40
'Okpo-mbontam	90±4.40	96±5.15

**Data are means and standard errors of five (5) replicates*

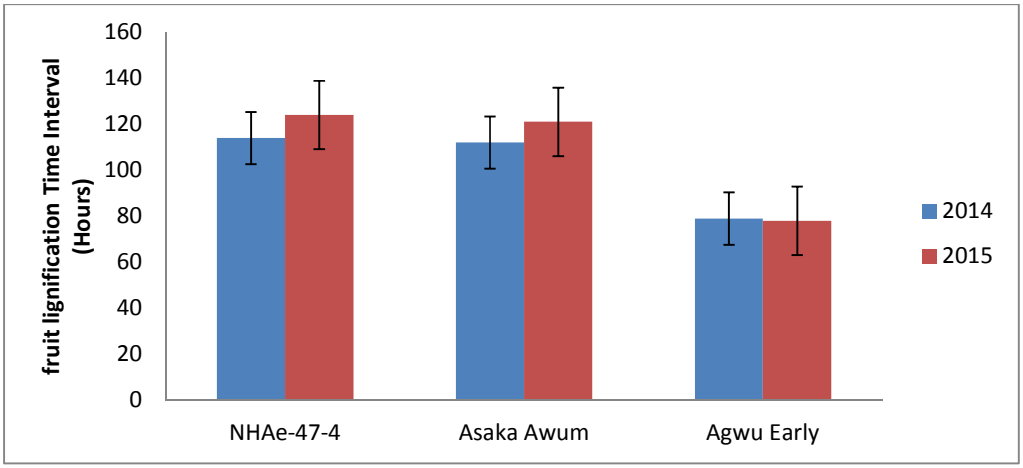

Fig. 7. Chart showing fruit lignification time interval (hours) for 3 dwarf early maturing okra genotypes

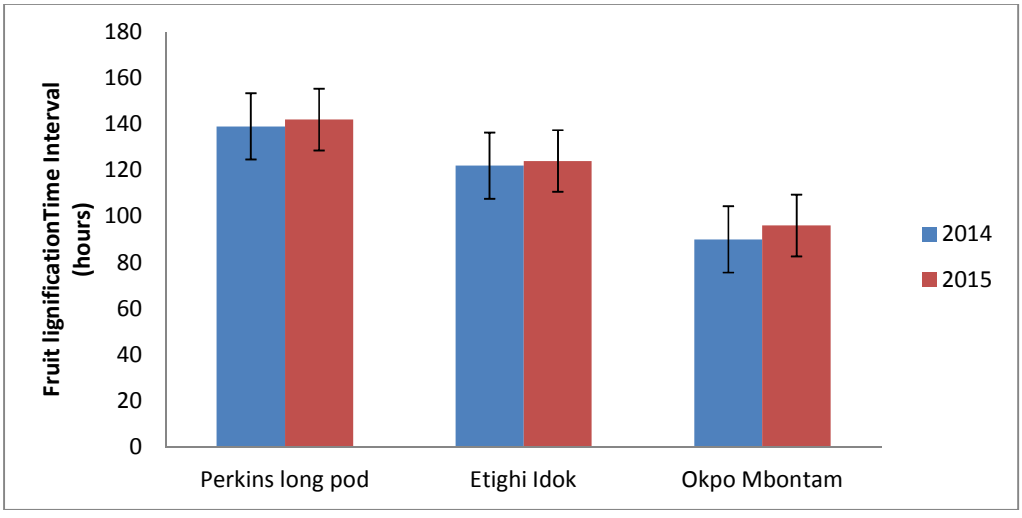

Fig. 8. Chart showing fruit lignification time interval (hours) for 3 tall late maturing okra genotypes

4. DISCUSSION

The study revealed that the tall, late maturing okra genotypes, Perkins long pod, 'Etighi Idok' and 'Okpo-mbontam' fruits required longer time interval to become lignified, had a longer life span and long period of fruiting compared to the short, early maturing okra genotypes NHAe-47-4, 'Asaka awum' and 'Agwu early' which showed shorter life span, shorter period of fruiting and required shorter time interval to became lignified. The study report finding is in line with the reports of [1,13,14,15].

Knowledge of fruit lignification time interval in Okra will no doubt reduce the laborious task of daily handpicking of immature fruits, reduce the rate of spoilage of the vegetable, enable for the consumption of fresh rather than dried okra and for plan and controlled harvesting of fruits for the market, income to the farmers and ensure food security.

The study was separated for early maturing and late maturing Okra genotypes due to the differences in their life span. The dwarf early maturing genotypes are ephemeral in nature (that is) short life span of not more than three months while the tall, late maturing genotypes are annual in their life span. This findings has revealed that the early maturing genotypes which are normally cultivated two or thrice a year in irrigated areas has shorter time interval to become unusable in their fresh conditions while the late maturing genotypes which are normally not often cultivated because of their long period of nurturing, has longer time interval for fruits to become lignified.

This calls for an improvement of the early maturing, dwarf genotypes in terms of prolonging their fruits lignification time interval so that it will yield more economic returns to the farmers. As breeders, the identification of the genes responsible for fruit lignification in Okra through the use of molecular markers and other marker assisted breeding and protocols is strongly advocated. The identification of the responsible gene(s) and the genetic engineering (isolation and stable incorporation of the desired gene(s) of interest) to the genome of other genotypes with short fruit lignification time interval, develop and distributes to farmers for use will reduce the wastage and loss associated with fruit lignification in Okra.

Also, studies are underway, to unveil the potentials of prolonging time interval for Okra fruit lignification, using local available materials as soil additive for this important biochemical and physiological process in Okra vegetable. This, when the results are out, will also help local farmers to always applied and make use of the best soil additive that would help prolong the fruit lignification time interval, thus bridging the gap between fruit yield and income loss due to lignification process.

The study tried to express the duration of time for dry matter or mineral accumulation in the fruits of okra which confers rigidity, hardiness and woodiness to the fruits making them unusable in fresh condition [5,16]. This phenomenon from our study is time dependent and hence the object of the study which had sought to identify the time required for the different genotypes studied [12,17].

The local okra genotypes Asaka awum, Agwu early, Etighi idok and Okpo-mbontam fruits lignified at a shorter time interval when compared to the improved genotypes NHAe-47-4 and the Perkins long pod. The phenomenon of fruit lignification in okra has remained a source of worry and concern to the local okra farmers in this agro-ecology and others. The phenomenon has been responsible for the undesired and underutilization of 'dried okra' which shows reduced nutritional and economic value when compared to fresh unlignified fruits [5,18].

Emphasis should be geared towards the improvement of the local okra genotypes 'Etighi idok', 'Asaka awum', 'Agwu early' and 'Okpo-mbontam' which are mostly used by the farmers as planting material in subsequent seasons [7,19]. Planned research efforts geared towards identifying the biochemical and physiological bioremediation using nutrient additives or any other soil or plant processes is strongly advocated if food security and sustainability as well as increasing farmer's income and yield are our targets. Planned breeding programmes targeted at prolonging fruit lignification interval for this nutritious vegetable fruit is advocated.

The present study seeks to provide baseline information on fruit lignification time interval in only few okra genotypes. It provides a stepping stone towards the development of predictive model which can help put an end to the wastage of this important vegetable fruits due to the lignification phenomenon in okra.

5. CONCLUSION

Fruits lignification time interval study in okra genotypes can be exploited by breeders, agronomists, Biochemist and horticultural for large-scale cultivation and production of okra through controlled and timely harvesting. Adequate marketing channels for fresh and unlignified okra fruits can also be explored before harvesting in other to dispose fresh fruits, which have very short shelf life. This is possible through the integration of various sources of knowledge of fruit lignification time intervals. Moreover, fresh okra fruits utilization can be enhanced and promoted through availability of more fresh and unlignified fruits in the markets instead of the unprocessed lignified 'dried Okra' fruits. Future recommendation on the most appropriate Okra genotypes for use by local farmers can be made based on this study. Further study should be multi-locations and include wide range of genotypes to overcome the yield and economic gaps.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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