## **Original Research Article**

# IMPACT OF HYBRIDIZATION ON OKRA (Abelmoschus esculentus) NUTRIENT *ENRICHMENT*

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#### 5 ABSTRACT

6 Studies have shown that significant differences exist in the morphological, Phytochemical and 7 nutrient characteristics among okra varieties. Plant breeders try to substitute the undesirable qualities 8 in plants with desirable ones so that it will result in higher yield of crops of improved quality and 9 this is achieved through the process of hybridization. In this study, hybridization was carried out on 10 two varieties of okra namely: Clemson spineless and dwarf long green to raise F<sub>1</sub> so as to assess the expression of heterosis or improved vigour and the impact of hybridization on okra nutrient 11 12 enrichment as shown in vitamin compositions (vitamin A, B<sub>1</sub>, B<sub>2</sub>, B<sub>3</sub> and C (fruits part) of the 13 parental plants compared with the F<sub>1</sub> hybrid. This was done using standard methods. Results were 14 analyzed using one way analysis of variance (ANOVA). Results showed that the parental and the  $F_1$ 15 plants contained varied quantities of all the investigated vitamins. Higher contents of Vitamins A, B<sub>2</sub> 16 and C were recorded for the F<sub>1</sub> hybrid when compared with the parental plants. So, there was an 17 expression of heterosis indicating that hybridization could greatly enriched vitamin nutrients in okra. 18 Moreover, F<sub>1</sub> generation combined the characters of Clemson Spineless and Dwarf long green 19 varieties and also had additional improved genetic attributes. This can be seen in most nutrients 20 being higher in the F<sub>1</sub> hybrid and also the reduced spines in the fruit of the F<sub>1</sub> hybrid as opposed to 21 the prominent spines in dwarf long green parent.

Keywords: hybridization, okra varieties, Clemson spineless, dwarf long green, heterosis, vitamin
 composition, nutrient enrichment

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#### **30 INTRODUCTION**

*Abelmoschus esculentus* L.(Moench), Okra, is an important vegetable in the family Malvaceae. It is a green fruit which is strictly used as a vegetable and is valued for its edible green seed pods containing round, white seeds (Jesus *et al*, 2008). It is an economically important vegetable crop cultivated in tropical, subtropical and warm temperate regions around the world (Vaughan and Geissler, 2009). The fruits are said to be shaped like a lady's fingers - one of its common names in British English. The flowers and upright plants give okra an ornamental value (Duzyaman, 1997).

Okra provides dietary fibres and distinct seed protein balanced in both lysine and tryptophan amino acids (unlike the proteins of cereals and pulses) thus, referred as "a perfect villager's vegetable (Camciuc *et al.*, 1998). Okra is an annual herb, 3 to 6 feet tall with a hibiscus-like flower, a deep branched taproot and succulent stem with scattered, stiff hairs. The pods of *Abelmoschus esculentus* have a unique flavour and texture and release slimy mucilage on cooking, which can be used to thicken sauces and add smoothness to soups. Its mature fruit and stems contain crude fibre, which is used in the paper industry. (Mabberley, 2008).

In the Eastern part of Nigeria, precisely Anambra, there are five varieties which are the most common. They are; Green emerald, Dwarf long green, the local long pod variety, perkins spineless and Clemson spineless. The two varieties which are the focus of this study are the Clemson spineless and the dwarf long green. The Clemson spineless is long, narrow and do not possess spines; while the dwarf long green variety is shorter, greater in diameter and possess spines. The Dwarf long green variety has a darker shade of green colour than the Clemson spineless (Van, 2005).

50 Vitamin is an organic compound and a vital nutrient that an organism requires in limited 51 amounts. The body can only manufacture three vitamins D, K and Biotin a B vitamin. The rest must 52 be obtained from dietary sources. Studies have shown that significant differences exist in the morphological, phytochemical, nutrient and anatomical characteristics among okra varieties (Van, 53 54 2005). Furthermore, the modern scientific method of crop improvement was necessitated by the 55 present demands of modern man for good quality food crops; crops with good taste, crops that 56 mature early, crops that contain most of the essential food nutrients and crops that can meet the immediate needs of man in terms of food and raw materials (Duzyaman, 2010; Ilodibia et al., 2014 57 58 and 2015). Plant breeders try to substitute the undesirable qualities in plants with desirable ones so 59 that it will result in higher yield of crops of improved quality and this is achieved through the 60 process of crossbreeding or hybridization. Hence, this research aimed at hybridizing two varieties of 61 okra Clemson spineless and dwarf long green to produce a hybrid that might combine the qualities of

the two varieties with the removal of their bad traits to meet the popular demands of the society.
Such a hybrid of the two varieties is likely to be of better quality and demand than either of the two
varieties. Accordingly, the problem and focus of the researcher is to hybridize the two common
varieties found in Nigeria and compare the vitamin contents of the hybrid and the parental plants
(Clemson spineless and the dwarf long green). It will cover the first filial generation in related
qualities.

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#### 69 MATERIALS AND METHODS

#### 70 Collection, identification of plant materials and study area

The seeds of the two varieties of *Abelmoschus esculentus* (Clemson spineless and the dwarf long green) used in this work were obtained between March – April 2016 and authenticated at the Agricultural development project (ADP), Kwata, Anambra state, Nigeria. The experiments were carried out at the Botany laboratory, Nnamdi Azikiwe University, Awka, Anambra state and Emery Biotechnology Laboratory, Ahia-Eke, situated in Umuahia, Abia State. The design of the study was pure experimentation of randomized complete block design.

#### 77 Materials and experimental procedure

78 Seeds of two varieties of okra (Clemson spineless and dwarf long green (Fig. 1), twenty (20) black 79 polythene bags, weighing balance, a pair of forceps, aluminium foil, animal manure and loam soil. 80 After the seeds were obtained, a viability test was carried out on them. This was done by soaking a 81 handful of each variety in a beaker containing distilled water for approximately 3 minutes. The seeds 82 that floated on the surface of the soil were discarded while those that sank to the bottom of the 83 beaker were sown in a transparent glass jar containing loamy soil. After a few days, germination occurred proving the seeds to be viable. A total of twenty black polythene bags were obtained, 84 85 perforated and filled with 5kg of loamy soil each. Ten of the polythene bags were labeled 'Clemson spineless' while the other ten were labeled 'Dwarf long green' which are the two varieties to be 86 87 studied. The seeds of each variety were broadcast accordingly on the soil surface (five seeds per bag). At different stages, the seedlings were pruned to two seedlings per bag when they were 12cm 88 89 high. Flowering of the dwarf long green variety started at eight weeks while that of the Clemson 90 spineless variety started at nine weeks. It was at this stage that the crossing or hybridization was

91 done. Artificial crossing was adopted because okra is a self-pollinating crop. This involved the 92 removal of anthers with a pair of forceps from the dwarf long green variety thus using it as a female 93 parent before it dehisces and covering it with a study bag (foil) to avoid natural crossing by insects. 94 This was followed by the collection and transference of ripe pollen grains from the Clemson 95 spineless (male parent) to the stigma of the emasculated plant (dwarf long green). This pollination 96 process was followed by fertilization and subsequent production of fruits (the  $F_1$  hybrid). This 97 procedure is as outlined by Ilodibia *et al.* (2014).

#### 98 Vitamin nutrient analysis

Dried fruits of two varieties of okra (Clemson spineless, Dwarf long green) and the  $F_1$  hybrid were ground into fine (100-mesh screen) powder with a dry mill and then examined for the following vitamins (A, B1, B2, B3 and C) using the standard methods described by AOAC (2005).

#### 102 STATISTICAL ANALYSIS

Data obtained was statistically analyzed using one way analysis of variance (ANOVA). The
 Duncan's multiple range test was used to test the difference among treatments at 0.05% level.
 Results were presented in Mean± Standard Error.

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#### 107 RESULTS AND DISCUSSION

108 Results of the study were shown in Table 1 and Figures 1-4.

Results showed that the parental and the  $F_1$  plants contained varied quantities of all the investigated vitamins except B3 which was absent in  $F_1$  (Table 1). This conforms to the report of Velu *et al.* (2011) and Ilodibia *et al.* (2015), that several hundred wheat accessions showed four to five fold variability for grain Fe and Zn concentrations and the two species of pepper (*Capsicum frutescens* and *C. annuum*) and F1 hybrid all contained different nutrient compositions respectively. The fruit of the dwarf long green variety contained higher quantities of Vitamins B<sub>1</sub> and B<sub>3</sub> when compared with the fruit of the Clemson spineless variety and the F<sub>1</sub> hybrid.

- 116 Higher contents of Vitamins A, B<sub>2</sub> and C were recorded for the F<sub>1</sub> fruit when compared with the
- fruits of parental plants. Vitamin A:  $8.75 \pm 0.00$ ,  $9.40 \pm 0.00$  and  $9.75 \pm 0.00$  for clemson spineless,
- 118 dwarf long green and F<sub>1</sub>hybrid respectively (Table 1, Fig. 1). Vitamin B: 0.45±0.00, 0.35±0.00 and
- 119 0.50 $\pm$ 00 for clemson spineless, dwarf long green and F<sub>1</sub> hybrid respectively (Table 1). Vitamin C:

120 18.80±0.14, 17.53±0.11 and 19.20±0.10 for clemson spineless, dwarf long green and F<sub>1</sub>hybrid respectively (Table 1). This is in line with the report of Allard (1960) that the progeny of 121 122 hybridization will combine many of the qualities of both parents thus producing a crop which is superior to some extent to either of the parents. Again, Nwakile (1994) reported that when the male 123 124 pollen of a plant is manually transferred, into the female stigma of another plant which may be of a 125 different variety, species or even genera, a hybrid with improved characters is produced. Also, with 126 that of Ilodibia et al. (2014 and 2015), who reported that F1 generation combined the characters of 127 both parents and other improved attributes.

128 Furthermore, the F<sub>1</sub> generation combined the characters of both the Clemson spineless and Dwarf 129 long green varieties, although the characters of the Dwarf long green dominated that of the Clemson 130 spineless variety. This can be seen in the reduced number of spines possessed by the F<sub>1</sub> hybrid as 131 opposed to the Clemson spineless which has no spines. This agrees with what Mendel (1866) pointed out that when two plants with pair of contracting characters are hybridized, one of the 132 133 characters would often appear in the offspring while the other remain masked. The character that 134 appeared in the offspring he called dominant character while the character that did not appear he 135 called recessive character. Vitamin A provides healthy skin, good eye sight, a powerful antioxidant 136 that protects against cancer and heart disease and enhances the immune system. Vitamin B1 provides healthy heart and nervous system, optimizes metabolism and brain formation. Vitamin B2 helps in 137 the formation of antibodies and red blood cells, facilitates carbohydrate, fat and protein metabolism, 138 139 aids against stress and fatigue. Vitamin C is an anti-stress vitamin, protects the cells from toxic 140 wastes and is the most powerful and effective of all the antioxidants, reduces infections, allergies, 141 asthma and common cold.

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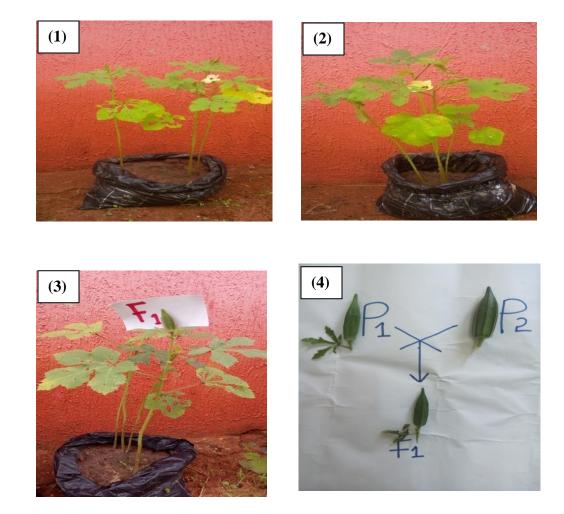
# Table 1: Mean Percent Vitamin compositions of the fruit of Clemson spineless, Dwarf long green and F<sub>1</sub>hybrid (%).

Parameters	Clemson spineless	Dwarf long green	F <sub>1</sub> Fruit	p-value
	(F)	(F)		
Vit. A (UI/100g)				
	$8.75 \pm 0.00^{a}$	$9.40{\pm}0.00^{ m b}$	$9.75 \pm 0.00^{\circ}$	0.01
Vit. B <sub>1</sub> (mg/100g)	$0.45 {\pm} 0.00^{b}$	$0.35{\pm}0.00^{a}$	$0.50{\pm}0.00^{\circ}$	0.05
Vit. B <sub>3</sub> (mg/100g)	$0.88{\pm}0.00^{b}$	1.04±0.00 <sup>c</sup>	$0.10{\pm}0.00^{a}$	0.00
Vit. C (mg/100g)	$18.80{\pm}0.14^{b}$	17.53±0.11 <sup>a</sup>	19.20±0.10 <sup>c</sup>	0.00

156 Results are in Mean ±Std.

157 The same letter in a column is not significantly different by Duncan's multiple range test at (p<0.05)

158 F: fruit



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- Fig. (1) The parental variety  $P_1$  (Clemson spineless) (2) Parental variety  $P_2$  (Dwarf long green) (3)  $F_1$ hybrid and (4) Fruits of parents compared with the fruit of the  $F_1$  hybrid.

- **Conflict of Interests**: Authors have declared that no competing interests exist.

182	CONCLUSION
183	Results of this study showed that the $F_1$ generation combined the characters of Clemson Spineless
184	and Dwarf long green varieties and also had additional improved genetic attributes. This can be seen
185	in most nutrients being higher in the $F_1$ hybrid and also the reduced spines in the fruit of the F1
186	hybrid as opposed to the prominent spines in dwarf long green parent. Thus hybridization could
187	greatly enriched vitamin nutrients in okra.
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192 193	Allard, R.W. (1960). <i>Principles of plant Breeding</i> . New York, John Wiley and Sons.
194	AOAC (Association of Official Analytical Chemists) (2005). Official Methods of Analysis of the
195	Association of Official Analytical Chemists International. Gaithersburg, Ontario Cnada. Pp.
196	2205-2314.
197	Camciuc, M., Deplagne, M., Vilarem, G. and Gaset, A. (1998). Okra - Abelmoschus esculentus L.
198	(Moench.) a crop with economic potential for set aside acreage in France. Industrial Crops
199	and Products 7: 257–264.
200	Duzyaman, E. (1997). Okra: botany and horticulture. Horticultural Review 21:42-68.
201	Duzyaman, E. (2010). Okra: botany and horticulture. Horticultural Reviews 21: 24-67.
202	Ilodibia, C.V., Okeke, N.F., Egboka, T.P., Achebe, U.A., and Chukwuma, U.M. (2014). Plant
203	breeding for food security sustainability and industrial growth. International Journal of
204	Plant Breeding and Genetics, 8: 219-223
205	Ilodibia, C.V., Ugwuoke, C.E., Egboka, T.P., Akachukwu, E.E., Chukwuma, U.M. and Aziagba,
206	B.O.(2015). Breeding pepper for enhanced food nutrients. Asian Journal of crop science
207	7(3): 214-218
208	Jesus, M. M., Carnelossi, M. A., Santos, S. F., Narain, N. and Castro, A. A. (2008). Inhibition of
209	enzymatic browing in minimally processed okra. Revista Ciencia Agronomica 39 (4): 524-
210	530.
211	Mabberley, D. J. (2008). Mabberley's Plant-book: a Portable Dictionary of Plants, their

212	Classification and Uses. 3rd Edition. Cambridge University Press, Cambridge, UK. Pp 59-98.
213	Mendel, G., 1866 Versuche uber p? Anzenhyriden. Verhandlungen Naturforschenden Vereines
214	Brunn, 4: 3-47
215	Nwakile, O. (1994) Modern Agricultural Sciences for Schools and Colleges. Nigeria, Medi
216	Commercial Agencies Limited.
217	Van, B. (2005). Food Plants of the World: An Illustrated Guide. Timber Press, Inc., Portland,
218	Oregon. Pp 32-52.
219	Vaughan, J. G. and Geissler, C. A. (2009). The New Oxford Book of Food Plants. 2nd Edition.
220	Oxford University Press, Oxford, UK. Pp 45-69.
221	Velu, G., Singh, R., Heurta-Espino, J., Pena, J. and Oritz-Monasterio, I. (2011). Breeding for
222	enhanced Zinc and Iron concentration in CIMMYT spring wheat germ plasm. Czech Journal
223	of genetics and plant breeding 47: 174-177.
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