Original Research Article

Improvement of fruit pubescence and fruiting pattern among pure lines and Landrace of Okra (*Abelmoschus* spp.) through interspecific hybridization

Abstract

The research study was aimed at improving fruit pubescence and growth pattern in the highly mucilaginous but very spiny local and wild okra variety - '*Ele Ogwu'* through generic hybridization with selected glabrous conventional and early maturing varieties at the Research Farm of the Department of Crop Science, University of Nigeria, Nsukka. The hybrid seeds generated from their diallel crosses were selfed to generate the F_2 seeds. Backcrosses (BC_1 and BC_2) were also made to the much improved and glabrous varieties. The parents, F_1, F_2 and BC genotypes were sown in an experimental field in a randomized complete block design and organoleptic assessment was done at harvest. The F_2 hybrids from the crosses, *'UHIE x CLM'* and *'AGW x CLM'*, yielded some smooth fruits; although most of the hybrids generated, exhibited intermediate smoothness, except for *'OGW x UHIE'* and *'OGW x LD88'* that exhibited high degree of spineness. Most of the F_2 hybrids generated exhibited intermediate growth and fruiting pattern, except for *'OGW x UHIE'* which showed indeterminate pattern. The Chi-square statistics for the F_2 showed that fruit pubescence for the highly spiny landrace and the more glabrous improved ones was monogenically controlled with incomplete dominance, while growth pattern were polygenically controlled. The successful backcrosses obtained showed reduction in the proportion of fruits spininess and increased fruit determinate growth pattern. This hereby indicates the prospect of obtaining okra plants with glabrous, highly appealing and determinate growth pattern with early maturity from the local, wild spiny but mucilaginous, nutritious and high yielding local varieties through sustained hybridization programme.

Keywords: *Abelmoschus* spp**.,** Interspecific hybridization, Fruit pubescence, Spininess, Varieties

INTRODUCTION

Okra (*Abelmoschus* spp.*)* is one of the most widely known and utilized crops of the family Malvaceae (Naveed *et al*., 2009) and an economically important vegetable crop grown in tropical and sub-tropical parts of the world (Oyelade *et al*., 2003; Andras *et al*., 2005; Saifullah and Rabbani, 2009). Okra originated in Ethiopia (Simmone *et al.*, 2004; Sathish and Eswar, 2013; Siemonsma and Kouamé, 2004). It is suitable for cultivation as a garden crop as well as on large commercial farms (Gemede *et al.*, 2014). It is widespread in tropical, subtropical and warm temperate regions, but is particularly popular in West Africa, India, the Philippines, Thailand and Brazil (Qhureshi, 2007). Okra production is estimated at 6 million tonnes per year in the world (Sorapong, 2012).

According to Mihretu *et al*. (2014), it is a multipurpose crop due to its various uses of the fresh leaves, buds, flowers, pods, stems and seeds. The immature fruits are mostly consumed as vegetables and can be further processed into salads, soups and stews. They can be consumed in their fresh or dried states; fried or boiled (Ndunguru and Rajabu, 2004). The very spiny local variety (Ele Ogwu), offers highly mucilaginous consistency after cooking compared to the more widespread conventional varieties (Maramag, 2013). Often, the extract obtained from the fruit is added to different recipes like soups, stews and sauces to increase the consistency. The mucilage has medicinal applications when used as a plasma replacement or blood volume expander (Madison, 2008; Maramag, 2013). The variety is also well suited and mostly adaptable to tropical regions with relatively high fruit yield (Udengwu, 2008).

However, the '*Ele Ogwu*' variety is characterized by the presence of spines and injurious hairs on the pods stems and leaves, otherwise known as trichomes. Trichomes are unicellular outgrowths from the epidermis of leaves, shoots and roots. It is evident that trichomes play a role in plant defence, especially with regard to phytophagous insects by reducing feeding capacity and oviposition (Nawab *et al*., 2011). According to Stiller *et al*. (2004), Pubescence phenotypes are described as smooth (no trichomes), hirsute (moderate pubescence) or pilose (dense pubescence). The spines or trichomes however affect consumer consumption and general acceptability of the fruit. Their presence makes picking an unpleasant job, although preferred by consumers, because they contain high percentage of mucilage (Abdelmageed, 2010). The inheritance of trichomes or spines on the surface of fruits has been found to be complicated by the fact that they can be seasonally and developmentally influenced in the sense that they appear non hairy at an early stage of development and hairy at a later stage (Kadams *et al.,* 2015). On the other hand, the dwarf and improved okra varieties (*Abelmoschus esculentus*) apart from being photoperiodically neutral or photoperiodically less sensitive, are much glabrous, with more appealing texture and ease of harvesting and processing (Udengwu, 2008; Mujeeb-Kazi and Rajaram, 2002; Abdelmageed 2010).

Inter specific hybridization has been mostly used for the transfer of specific characters such as disease and pest resistances as well as determining the inheritance pattern of various qualitative traits such as fruit colour, spineliness *etc* from related species to cultivated species (Prabu and

Warade, 2013). Hybridization involving wild and cultivated varieties has long been used for transfer of genetic material to the crops. A promising breeding method for creation of glabrous or less spiny fruits has become a major topical issue towards enhancing the utilization and consumption of okra

Hence, the study was aimed at investigating the mode of inheritance and genetic improvement of fruit pubescence of the very spiny '*Ele Ogwu*' variety, so as to improve its utilization as well as meeting the ever increasing demands for the crop.

MATERIALS AND METHODS

The experiment was carried out in between 2013 and 2014 at the Research farm of Department of Crop Science, University of Nigeria, Nsukka, Nigeria. Nsukka is located on latitude 06° North, longitude 07°24 East and altitude 447.26 m above sea level in the derived savanna of the South Eastern Agro-ecological zone of Nigeria (Uguru *et al.*, 2011). The genetic materials consisted of a local cultivar and landrace (*Ele Ogwu*) and three improved and dwarfish varieties (*Agwu early*, *Clemson spineless* and *LD 88*) as described in Table 1. The local cultivar was sourced from indigenous local farmers in Nsukka, Enugu State, while the improved varieties were sourced from NIHORT (National Institute for Horticultural Research and Training), Okigwe, Imo State, both in Nigeria.

Table 1: Description of the local and improved accessions of okra used for the study.

Accessions	**Type of cultivar**	**Sources of collection**	**Description of materials**
Ele Ogwu (OGW)	Local	Obukpa, Nsukka, Enugu State.	Dark green, highly mucilagious and very spiny
Agwu early (AGW)	Improved, conventional	NIHORT, Okigwe, Imo State	Dark green, slightly mucilaginous and less

			glabrous
Clemson spineless (CLM)	Improved, conventional	NIHORT, Okigwe, Imo State	Pale green, slightly mucilaginous and very smooth
LD88	Improved, conventional	NIHORT, Okigwe, Imo State	Dark green, slightly mucilaginous and partially glabrous

NIHORT = National Institute for Horticultural Research and Training

Twelve plots measuring 8 m x 5 m were prepared and each accession allotted to a plot comprising four stands. Planting was done at a spacing of 0.5 m x 0.6 m. Two to three seeds were drilled per hole and later on thinned down to two vigorous plants per stand at 14 days after planting. Well cured poultry manure was incorporated at the rate of 8.65 tonnes/ha a week before bed preparation. Inorganic compound fertilizer was applied at the rate of 30 kg N/hectare at two separate doses (two weeks after planting and at the onset of flower bud). Weeding was done manually before flowering followed by rouging, which was done during flowering to keep weed pressure low.

At flowering, the accessions were crossed in all possible combinations to produce F_1 hybrids. In the second period of planting, the hybrids and parents were sown out in plots measuring 6 m x 4 m. The hybrids were selfed to produce the F_2 seeds and also backcrossed to the okra parents to produce the backcrosses (BCs). In the third planting, the parents, F_1, F_2, and BCs were sown out separately in beds measuring 6 m x 5 m in a randomized complete block design (RCBD). At maturity, fruits were harvested per plant in each plot. Fruit from each plant during the period of harvesting, was carefully felt with the fingers as well as through careful observation to ascertain the degree of pubescence or spininess of each fruit. Numerical counts were then taken on each plot on the different degrees of pubescence or spininess. Genetic ratios were tested using the Chi-square (χ^2) statistic. The Yates correction for continuity for Chi-square (Stansfield, 1969) was also used especially for those with unitary degrees of freedom.

RESULTS AND DISCUSSION

The presence of spines or trichomes especially on the fruit pods of okra has really been a limiting factor towards advanced production of okra. Interspecific hybridization with compatible improved varieties have proven instrumental in reducing the appearance of spines (Kadams *et al.*, 2015). From studies, a single gene was found to be responsible for the inheritance of spines on pods but with incomplete dominance. Abdelmajeed (2010) opined that fruit spininess was monogenically controlled with the absence of complete dominance of one allele over the other. Furthermore, maternal effects arise where the mother makes a contribution to the phenotype of her progeny over and above that resulting from genes she contributes to the zygote (Mather and Jinks, 1974). Results in Table 4 showed that a sizable proportion of the F_1 following the interspecific hybridization between local okra cultivar (*Abelmoschus caillei*) and improved okra cultivar (*Abelmoschus esculentus*) produced hybrids with very smooth fruits; *'OGW x CLM'*, except *'OGW x AGW'* and *'OGW x LD88'* crosses that produced genotypes that were intermediately smooth (relatively smooth and spiny) and some degree of spineness. The success recorded in the production of F_1 genotypes from the generic crossability between local okra and improved okra cultivars underlies prospects for improved fruit pubescence in local cultivars via improved ones. Although few attempts at crossing the mostly spiny local cultivars with smooth cultivars have been recorded, however Abdelmageed (2010) in his study opined that, the degree of success was dependent on the direction of the cross. Furthermore, he revealed that the desirable spineless fruits with other desirable characters can be attained through hybridization and selection in the segregating generations. However, he suggested that experiments were needed at early and late season to confirm the mode of inheritance of this trait. The production of intermediate smooth fruits in all the hybrids (F_1); *'OGW x AGW'* and *'OGW x LD88'* except *'OGW x CLM'* showed partial dominance of allele for fruit pubescence over the allele for spineness. The dominance of gene controlling fruit pubescence played a significant role. However, the very smooth fruits obtained in the hybrid, *'OGW x CLM'* suggested complete dominance could be attainable.

The results observed in F_2 progenies of these hybrids showed that *'OGW x CLM'* produced genotypes in the following categories; slightly prickly = 9, very smooth= 14 and intermediate smooth = 33 (Table 2), while the cross, *'OGW x AGW'* produced fairly smooth fruits in the categories; very prickly = 25, intermediate smooth = 12 and slightly prickly = 33 (Table 4). Unlike the initial result, F_2 progenies of *'OGW x LD88'* (Table 6) gave the highest proportion of genotypes with intermediate fruit pubescence (very prickly = 33, intermediate smooth = 17 and slightly prickly = 31). The Chi-square estimates to test the goodness of fit of the genotypic ratio obtained to the Mendelian ratio are shown in Tables 3, 5, and 7. Significant differences ($P < 0.05$) between the expected (1:2:1) and observed ratios were observed in all the crosses. The test showed that the F_2 segregates for fruit pubescence in the hybrids developed did not exhibit significant difference ($P < 0.05$) between the expected (1:2:1) and observed ratios except for *'OGW x AGW'*. This is an indication that the observed ratio fit into the expected (Mendelian) ratio with probability value ranging from 0.25 to 0.01 for the hybrids except in *'OGW x AGW'*. The non-significant differences observed in most of the hybrids showed that fruit pubescence of the spiny local okra cultivars did not deviate from the Mendelian pattern of inheritance for the trait. This reveals that fruit pubescence existing in the cross between the spiny local cultivar (*A. caillei*) and the improved varieties (*A. esculentus*) is under the control of a single gene, i.e monogenically controlled. This agrees with the reports of Abdelmageed (2010) and Kadams *et al*. (2015) that, a single gene was found to be responsible for the inheritance of spines on pods but with incomplete dominance *i.e.* the character of spiny pods is partially dominant over the glabrous pods.

The successful backcrosses showed that all the hybrids obtained had high proportion of genotypes with intermediate smooth fruits, i.e fairly pubescent; *'OGW x AGW'* (very prickly = 25, intermediate smooth = 19, slightly prickly = 29) and *'OGW x LD88'* (very prickly = 11, intermediate smooth = 24, slightly prickly = 47) as shown in Tables 4 and 6 respectively. The highest proportions of intermediate smoothness and overall fruit pubescence was obtained in the cross *'OGW x CLM'* (slightly prickly = 5, very smooth = 23, intermediate smooth = 47). This segregation however does not fit into the digenic ratio of 1:2:1 (Table 4).

Table 2: Phenotypic Expression of Fruit pubescence in cross *OGW* (very prickly) x *AGW* (intermediate smooth) Okra genotypes

Fruit pubescence	P_1(OGW)	P_2(AGW)	F_1	F_2	BC_1	BC_2	TOTAL
Very prickly	78	0	0	25	45	27	**175**

Intermediate smooth	0	68	0	12	0	19	**99**
Slightly prickly	0	0	61	33	18	29	**141**
TOTAL	**78**	**68**	**61**	**70**	**63**	**75**	**415**

Table 3: Chi-Square Estimate of Fruit pubescence in F_2 progenies progenies in cross *OGW* (very prickly) x *AGW* (intermediate smooth) Okra Genotypes

Fruit pubescence	Observed (O)	Expected (E)	(O-E)	$(O\text{-}E)^2$	$(O\text{-}E)^2/E$
Very prickly	25	17.5	7.5	56.25	3.21
Intermediate smooth	12	35	-23	529	15.11
Slightly prickly	33	17.5	15.5	240.25	13.73
TOTAL	**70**	**70**	**0**	**825.5**	**32.05**

Expected ratio = 1:2:1 **χ^2 = 32.05**

Probability value for χ^2 = 0.25-0.10

Table 4: Phenotypic Expression of Fruit Pubescence in cross *OGW* (Very prickly) x *CLM* (Very Smooth) Okra genotypes

	PHENOTYPES						
Fruit Pubescence	**P_1(OGWU)**	**P_2(CLEMSON)**	**F_1**	**F_2**	**BC_1**	**BC_2**	**TOTAL**
Slightly prickly	78	0	0	9	19	5	**111**
Very smooth	0	71	0	14	0	23	**108**
Intermediate smooth	0	0	69	33	42	57	**201**
TOTAL	**78**	**71**	**69**	**56**	**61**	**85**	**420**

Table 5: Chi-Square Estimate of Fruit Pubescence in F_2 progenies progenies in cross *OGW* (Very prickly) x *CLM* (Very smooth) Okra Genotypes

Fruit pubescence	Observed (O)	Expected (E)	(O-E)	$(O\text{-}E)^2$	$(O\text{-}E)^2/E$
Slightly prickly	9	14	-5	25	1.79
Very smooth	14	14	0	0	0
Intermediate smooth	33	28	5	25	0.89

TOTAL	**56**	**56**	**0**	**50**	**2.68**

Expected ratio = 1:2:1 χ^2 **= 2.68**
Probability value for χ^2 **= 0.25-0.10**

Table 6: Phenotypic Expression of Fruit pubescence in crosses *OGW* (Very prickly) x *LD88* (intermediate Smooth) Okra genotypes

		PHENOTYPES					
Fruit Pubescence	**P_1(OGWU)**	**P_2(LD88)**	**F_1**	**F_2**	**BC_1**	**BC_2**	**TOTAL**
Very prickly	78	0	0	23	21	11	**133**
Intermediate smooth	0	85	0	17	4	24	**130**
Slightly prickly	0	0	57	31	36	47	**171**
TOTAL	**78**	**85**	**57**	**71**	**61**	**82**	**434**

Table 7: Chi-Square Estimate of Fruit pubescence in F_2 progenies progenies in cross *OGW* (Very prickly) x *LD88* (Slightly smooth) Okra Genotypes

Fruit pubescence	**Observed (O)**	**Expected (E)**	**(O-E)**	**$(O-E)^2$**	**$(O-E)^2/E$**
Very prickly	23	17.75	5.25	27.56	1.55
Slightly smooth	17	17.75	-0.75	0.56	0.03
Slightly prickly	31	35.50	-4.50	20.25	0.57
TOTAL	**71**	**71**	**0**	**48.37**	**2.15**

Expected ratio = 1:2:1
χ^2 **= 2.15**
Probability value for χ^2 **= 0.25-0.10**

The result showed partial dominance (*OGW x CLM*) of smoothness over spiny forms, thus; underlining the fact that backcrossing would be an important conventional breeding method for introgression of the trait of fruit pubescence, especially in local okra cultivars. Robbins (2012) earlier stated that backcross breeding is an effective method to transfer one or a few genes controlling a specific trait from one line into a second, usually elite breeding. Therefore, sufficient backcrossing to the more conventional pubescent cultivars can be used as an efficient method of breeding for reduced fruit spininess in okra.

From the results obtained, there was compatibility in the hybridization of local okra cultivar (*Abelmoschus caillei*) and improved okra varieties (*Abelmoschus esculentus*). Positive improvement in reducing the fruit spininess in *'Ele Ogwu'* was obtained through hybridization with an improved cultivar, *'Clemson spineless.'* Other form of improvement was observed in the cross, *'OGW x LD-88'* which gave moderate smooth fruits (F_2 = 17, BC_1 = 4 and BC_2 = 24 out of a plant population of 130 stands). The Chi square statistic showed that fruit pubescence in local okra cultivar (*A. caillei*) and improved okra cultivar (*A. esculentus*) cross was under the control of a single gene. The successful backcrosses obtained in the cross, showed that backcrossing would be an important conventional breeding method for introgression of fruit pubescence in okra especially in highly mucilaginous but spiny local cultivars (Abdelmageed, 2010; Nath and Dutta, 1970).

CONCLUSION

The relatively lower proportion of very spiny fruits obtained compared to those that were very smooth and intermediately smooth in F_1, F_2 and BCs is an indication of prospect in the improvement of fruit pubescence of the highly mucilaginous but spiny local cultivar through inter hybridization. The selection of more glabrous, improved varieties, *'Clemson spineless'* and *'LD-88'* for hybridization with the more spiny landraces have proven useful in achieving the aim of improving fruit pubescence which would influence improved consumption, especially in West Africa, that a larger part of the population craves for highly mucilaginous soups and other related dishes. Therefore, further selection and improvement studies with these improved genotypes could prove pivotal in improving many landraces of okra whose genetic resources have not yet been fully tapped. Similarly, the prospects of acceptance and marketability of the crop would be enhanced through successful subjection of the trichomic trait inherent in most landraces through interspecific hybridization with especially *'Clemson spineless'*.

ACKNOWLEDGMENT

We thank the Department of Crop Science, University of Nigeria, Nsukka, Enugu State, Nigeria for providing the facilities for this research. Gratitude also goes to the National Institute for Horticultural research and training, Okigwe for supplying the seeds used for the experiment.

Special thanks also go to distinguished breeders; Prof. Michael I. Uguru, Prof. Peter E. Ogbonna and Mr. Noble U. Ukwu for their advisory contributions and unlimited assistance in the experimental design and field experiment. Academic and financial contributions of senior colleagues in related field of discipline are greatly appreciated

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