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Improvement of fruit taste quality of 'egusi' melon (*Colocynthis citrullus* L.) through hybridization with watermelon (*Citrullus lanatus* Thumb.)

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Abstract

The research was aimed to improve fruit taste in 'egusi' melon through generic hybridization with watermelon. Three accessions of 'egusi' melons (Egu) seeds were crossed separately with two varieties of watermelons; Kolos (WM 1) and Kolack (WM 2) to generate hybrids (F_1) that were later selfed (F_2). Backcrosses (BC) were also made to the watermelon parents. The parents, F_1, F_2 and BC genotypes were planted in the field in a randomized complete block design and organoleptic assessment was done at harvest. The F_1 produced only hybrids with sweet fruit taste, WM 1 x Egu 3 (26), Egu 1 x WM 2 (24) and Egu 3 x WM 2 (33) except Egu 1 x WM 1 that produced only genotypes with intermediate fruit taste (32). The Chi-square statistics for the F_2 showed that fruit taste in 'egusi' melon and watermelon cross was under the control of many genes. The successful backcrosses obtained showed reduction in the proportion of with fruits bitterness viz., Egu 2 x WM 2 (bitter= 0 and sweet= 10) and Egu 3 x WM 2 (bitter= 13 and sweet= 16) indicating prospect for improving the fruit taste quality of 'egusi' melon through hybridization with watermelon.

Keywords: Crossability, Cucurbitacin, 'Egusi'melon, Fruit quality, Inheritance pattern, Watermelon

Introduction

Bitter fruits are found in many plants mostly of the *Cucurbitaceae* family (Mashear, 2007). Fruits of some members of this family are important vegetables and seed crops having considerable economic value (Mashear, 2007) such as 'egusi' melon (*Colocynthis citrullus*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativa*) and pumpkin (*Telfeiria occidentalis*). The *colocynthis* is a small genus of 4 to 5 species in Africa, one of which is the *Colocynthis citrullus* ('egusi' melon) with basic chromosome number of 2n=22; n =11 (Ogbonna, 2013). 'Egusi' melon is a perennial herb, trailing naturally and indigenous to Arabia, West Asia, Tropical Africa and Mediterranean regions (Pravin et al., 2013). Kernels of its seeds can be eaten individually as snacks when roasted and used extensively for cooking purposes, either as a soup additive or as cooking oil source (Bande et al., 2012). The melon seeds contain 38% protein and 30 - 50% of semi drying oil (Olaniyi and Tella, 2011). Recently, it has been proved to be a feed-stock for bio-fuel (Solomon et al., 2010).

The bitter fruit taste in egusi renders its pulp (mesocarp) inconsumable due to the concentration of cucurbtacins (Gry et al., 2006). Cucurbitacins are a group of bitter tasting oxygenated tetracyclic triterpenes that are produced in the family Cucurbitaceae and other plant families. The natural roles of the compound in plants are probably related

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to defence against pathogens and pests (Davidovich-Rikanti et al., 2015). Some cucurbitacins also have considerable pharmaceutical values, since they possess anti-cancer, anti- inflammatory and cytotoxic properties (Recio et al., 2012; Chen et al., 2005). Although this compound has a wide range of pharmacological implications, its extreme bitterness in plants makes the crops inconsumable (Zhang et al., 2013; Gry et al., 2006). Contrary wise, watermelon (*Citrullus lanatus*) that has the same chromosome number to ‘egusi’ melon is known to possess little percentage of cucurbitacin compounds, thus making the pulp palatable for consumption. The production of cucurbitacin is mainly determined by genetic factors; however, environmental conditions can also affect their production (Kano and Goto, 2003). Davidovich-Rikanti et al. (2015) underlined that a dominant gene (Bi) governs fruit bitter taste in wild *C. colocynthis*. Fell (2014) stated that bitterness is known to be controlled by two genetic traits, “Bi” which confers bitterness on the whole plant, and “Bt,” which leads to bitter fruit in wild cucurbits, the family that includes melon, cucumber, pumpkin, watermelon and squash. Shang et al. (2014) also reported transcription factors Bi and Bt that regulate the pathway of curcubitacin in leaves and fruits of cucumber, respectively.

However, little or no online published research work has been done on the inheritance pattern of bitter fruit taste in ‘egusi’ melon. The knowledge from the inheritance pattern will help to breed for reduced bitterness in ‘egusi’ melon so that both the seeds and pulp will be consumed. Therefore, the objectives of this research were to study the crossability between ‘egusi’ melon and watermelon and to underpin the inheritance pattern of bitter fruit taste in ‘egusi’ melon that will ultimately pave way for breeding of ‘egusi’ melon with sweet fruit pulp.

Materials and Methods

The experiment was carried out in between 2013 and 2014 at the Research Field of Department of Crop Science, University of Nigeria, Nsukka, Nigeria. Nsukka is located on latitude 06^o N, longitude 07^{o}24 East and altitude 447.26 m above sea level in the derived savanna of the South Eastern Agro-ecological zone of Nigeria. The genetic materials consisted of ‘egusi’ melon (3 accessions) and watermelon (2 accessions) as described in Table 1, obtained from the Institute of Agricultural Research (IAR), Ahmadu Bello University, Zaria, Nigeria. Six plots each measuring 4 m x 3 m was prepared and each accession allotted per plot. Planting was done at a spacing of 1 m x 1 m. Three to four seeds were drilled per hole and later on thinned down to two vigorous plants per stand at 14 days after planting. Inorganic compound fertilizers N: P: K 15: 15: 15 was applied at the rate of 400 kg hectare^{-1} two weeks after planting and weeding. 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 season, the hybrids and parents were planted out in plot measuring 4 m x 4 m. The hybrids were selfed to produce the F_2 seeds and also backcrossed to the watermelon parents to produce the backcrosses (BCs). In the third planting, the parents, F_1, F_2, and BCs were planted out separately in beds measuring 4 m x 2 m in a randomized complete block design (RCBD). At maturity, fruits were harvested per plant in each plot. Fruit from each plant was cut open and the mesocarp was tried organoleptically to determine the fruit taste. Numerical counts were then taken on each plot on the different mesocarp tastes. Genetic ratios were tested using the Chi-square (X^2) statistic. The Yates correction for continuity for Chi-square (Stansfield, 1969) was also used.

Results and Discussion

Tables 2, 4 and 6 showed that all the F_1 following the crossing between ‘egusi’ melon and watermelon produced hybrids with sweet fruit taste; WM 1 x Egu 3 (26), Egu 1 x WM 2 (24) and Egu 3 x WM 2

Table 1. Description of 'egusi' melon accessions and watermelon varieties used for the study.

Accessions	Melon	Source	Description of Materials
Egu 1	'Egusi' melon	Taraba State	Thin skin and thick tips.
Egu 2	'Egusi' melon	Ebonyi State	Thin skin and black molded edges.
Egu 3	'Egusi' melon	Enugu State	Thin skin flat and oval shape
WM 1	Watermelon	IAR, Kaduna State	Thick brown skin, with dark patches
WM 2	Watermelon	IAR, Kaduna State	Thick brown skin with dark tips

IAR-Institute of Agricultural Research, Ahmadu Bello University, Samaru Zaria, Nigeria.

Table 2. Segregation of genotypes in the cross between WM 1 x Egu 3 parents modified

	Phenotypes		Genotypes		
(Fruit Tastes)	P_1 (WM 1)	P_2 (Egu 3)	F_1	F_2	BC
Bitter	0	28	0	0	-
Sweet	12	0	26	15	-
Intermediate	0	0	0	2	-
Total	12	28	26	17	-

P_1= first parent, P_2= second parent, F_1 = first filial generation, F_2= second filial generation, BC = backcross to watermelon (Kol) parent, - = no value.

(33) except in Egu 1 x WM 1 cross (Table 9) that produced only genotypes (32) with intermediate fruit taste (relatively sweet and bitter). The success recorded in the production of F_1 genotypes from the generic crossability between 'egusi' melon and watermelon underlies prospects for fruit taste improvement in 'egusi' melon via watermelon. Although few attempts at crossing sweet dessert watermelon and 'egusi' have been described, there are two reports indicating that degree of success was dependent on the direction of the cross (Gusmini et al., 2004). Genomic sequencing has revealed that the citron, egusi and dessert watermelons differ significantly in genome organization (Guo et al., 2013; Reddy et al., 2013; Chomicki and Renner, 2015). The production of sweet taste fruits in all the hybrids (F_1); WM 1 x Egu 3, Egu 1 x WM 2 and Egu 3 x WM 2 except Egu 1 x WM 1 showed complete dominance of allele for sweet fruit taste over the allele for bitter taste. The dominant gene controlling sweet taste played an important role. However, the intermediate fruit taste obtained in the hybrid, Egu 1 x WM 1 suggested partial dominance could be possible.

The results observed in F_2 progenies of these hybrids showed that WM 1 x Egu 3 produced genotypes in the following categories; bitter = 0, sweet= 15 and intermediate = 2 (Table 2), Egu 2 x WM 2 produced; bitter= 3, sweet= 21 and intermediate = 4 (Table 4) and Egu 3 x WM 2 (bitter= 0, sweet= 25 and intermediate= 19) (Table 6). Unlike other results, F_2 progenies of Egu 1 x WM1 (Table 9) recorded the highest proportion of genotypes with intermediate fruit taste (bitter= 1, sweet= 10 and intermediate = 16). The Chi-square estimates to test the goodness of fit of the genotypic ratio obtained to the Mendelian ratio are depicted in Tables 3, 5, 7, 8 and 9. Significant differences ($P<0.05$) between the expected (3:1) and observed ratios were observed in all the crosses except WM 1 x Egu 3 (Table 3). The test showed that the F_2 segregates for fruit taste in all the hybrids exhibited significant difference ($P<0.05$) between the expected (3:1) and observed ratios except in WM 1 x Egu 3. This is an indication that the observed ratio did not fit into the expected (Mendelian) ratio with probability value ranging from 0.01 to 0.001 for all the hybrids except in WM 1 x Egu 3. The significant differences observed showed that fruit taste of 'egusi' melon deviated from the Mendelian pattern of inheritance. This reveals that fruit taste in egusi melon and watermelon cross is under the control of many genes. Fell (2014) reported that two researchers at

Table 3. Chi-square estimates of F_2 progenies for fruit taste in WM 1 x Egu 3 crosses

Phenotypes Fruit Taste	Observed(O)	Expected(E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
Sweet	15	12.75	2.25	5.06	0. 4
Intermediate	2	4.25	-2.25	5.06	1.19
Total	17	17	0	10.12	1.59

Expected Ratio = 3:1 , X^2 = 1.59, Corrected = 2.02, Probability Value = 0.20-0.10

Table 4. Segregation of genotypes in the cross between Egu 2 x WM 2 parents added

	Phenotypes		Genotypes		
(Fruit Tastes)	P_1 (Egu 2)	P_2 (WM 2)	F_1	F_2	BC
Bitter	12	0	0	3	0
Sweet	0	4	24	21	10
Intermediate	0	0	0	4	9
Total	12	4	24	28	19

P_1= first parent, P_2= second parent, F_1 = first filial generation, F_2= second filial generation, BC= backcross to watermelon parent

Table 5. Chi-square estimate of F_2 progenies for fruit taste in Egu 2 x WM 2 crosses

Phenotypes Fruit Taste	Observed(O)	Expected(E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
Bitter	3	7	-4	16	2.29
Sweet	21	14	7	49	3.5
Intermediate	4	7	-3	9	1.29
Total	28	28	0	74	7.07

Expected Ratio = 1:2:1 , X^2 =7.07, Probability Value = 0.05-0.01

Table 6. Segregation of genotypes in the cross between Egu 3 x WM 2 parents added

	Phenotypes		Genotypes		
(Fruit Tastes)	P_1 (Egu 3)	P_2 (WM 2)	F_1	F_2	BC
Bitter	12	0	0	0	13
Sweet	0	4	33	25	16
Intermediate	0	0	0	19	10
Total	12	4	33	44	39

P_1= first parent, P_2= second parent, F_1 = first filial generation, F_2= second filial generation, BC = backcross to watermelon parent (Koalack).

Table 7. Chi-square estimate of F_2 progenies for fruit taste in Egu 3 x WM 2 crosses

Phenotypes Fruit Tastes	Observed(O)	Expected(E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
Sweet	25	33	-8	64	1.94
Intermediate	19	11	8	64	3.37
Total	44	44	0	128	5.31

Expected Ratio = 3:1, X^2 = 5.31, Corrected X^2 = 7.30, Probability Value = 0.01-0.05

the Chinese Academy of Agricultural Sciences and their colleagues identified nine genes involved in synthesis of cucurbitacin in curcubits and can be traced to the two transcription factors (Bi and Bt)

Table 8. Segregation of genotypes in the cross between Egu 1 x WM 1 parents for fruit taste.

	Phenotypes		Genotypes		
(Fruit Tastes)	P_1 (Egu 1)	P_2 (WM 1)	F_1	F_2	BC
Bitter	35	0	0	1	-
Sweet	0	12	0	10	-
Intermediate	0	0	32	16	-
Total	35	12	32	27	-

P_1= first parent, P_2= second parent, F_1 = first filial generation, F_2= second filial generation, BC = backcross to watermelon parent, - = no value.

Table 9. Chi-square estimate of F_2 progenies for fruit taste in Egu 1 x WM 1 crosses

Phenotypes Fruit Taste	Observed(O)	Expected(E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
Bitter	1	6.75	-5.75	33.06	0.57
Sweet	10	6.75	3.25	10.56	1.57
Intermediate	16	13.5	2.5	6.25	0.46
Total	27	27	0	49.87	6.93

Expected Ratio = 1:2:1, X^2 = 6.93, Probability Value = 0.03

earlier reported for the compounds. Zhang et al. (2012) also pinpointed that bitterness in cucumber fruit trait has a complex inheritance mechanism.

The successful backcrosses showed that highest proportion of genotypes with sweet fruit were obtained in Egu 2 x WM 2 (bitter= 0 sweet= 10 and intermediate= 9) and Egu 3 x WM 2 (bitter= 13, sweet= 16 and intermediate= 10) as showed in Tables 4 and 6 respectively. The result showed that greater proportion of the progenies were sweet in fruit taste as obtained in hybrids Egu 2 x WM 2 (bitter= 0, intermediate= 9, and sweet= 10) and Egu 3 x WM 2 (bitter = 13, intermediate =10, and sweet= 16). This segregation does not fit into the digenic and mongenic ratio of 1:2:1 and 3:1 (Table 4 and 6), respectively. The result showed partial dominance (Egu 2 x WM 2) and complete dominance (Egu 3 x WM 2) of sweet fruit taste over biter fruit taste underpinning that backcrossing would be an important conventional breeding method for introgression of fruit sweetness trait in ‘egusi’ melon. 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 sweet fruit taste can be used as an efficient method of breeding for reduced bitter fruit taste in ‘egusi’ melon.

From the results obtained, there was compatibility in the hybridization of “egusi” melon and watermelon. Positive improvement in reducing the fruit bitterness in ‘egusi’ melon was obtained through hybridization with watermelon. The Chi-square statistics showed that fruit taste in ‘egusi’ melon and watermelon cross was under the control of multiple genes. The successful backcrosses obtained in two crosses showed that backcrossing would be an important conventional breeding method for introgression of fruit sweetness trait in ‘egusi’ melon. Conclusively, the lower proportion of fruits with bitter taste obtained compared to those with sweet fruit taste in F_1, F_2 and BCs is an indication of prospect in the improvement of fruit taste of ‘egusi’ melon through hybridization with watermelon which will lead to consumption of not only the seeds, but also the pulp.

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