Journal of Heredity 2004:95(3):268–270
DOI: 10.1093/jhered/esh031

Inheritance of Egusi Seed Type in Watermelon

G. Gusmini, T. C. Wehner, and R. L. Jarret

From the Department of Horticultural Science, North Carolina State University, Raleigh, North Carolina (Gusmini and Wehner) and USDA-ARS, Plant Genetic Resource Unit, 1109 Experiment St., Griffin, GA 30223 (Jarret).

Address correspondence to T. C. Wehner at the address above, or e-mail: todd_wehner@ncsu.edu.

An unusual seed mutant in watermelon (*Citrullus lanatus* var. *lanatus*) has seeds with a fleshy pericarp, commonly called egusi seeds. The origin of the phenotype is unknown, but it is widely cultivated in Nigeria for the high protein and carbohydrate content of the edible seeds. Egusi seeds have a thick, fleshy pericarp that appears during the second to third week of fruit development. We studied the inheritance of this phenotype in crosses of normal seeded Charleston Gray and Calhoun Gray with two plant introduction accessions, PI 490383w and PI 560006, having the egusi seed type. We found that the egusi seed type is controlled by a single recessive gene, and the symbol *eg* was assigned.

The cultivated watermelon is classified as *Citrullus lanatus* (Thunb.) Matsum. & Nakai var. *lanatus*. The genetics of watermelon have been widely studied, and several genes have been characterized (Cucurbit Gene List Committee 1979, 1982; Henderson 1991, 1992; Rhodes and Dane 1999). Many genes controlling seed characters in this crop have been identified and their segregation patterns studied (Abd el Hafez et al. 1981; Kanda 1951; Kang et al. 2000; Poole et al. 1941; Porter 1937; Sharma and Choudhury 1982; Tanaka et al. 1995; Zhang 1996; Zhen Qing and Jin Hua 1995).

Some watermelon accessions in the U.S. Department of Agriculture-Agricultural Research Service (USDA-ARS) germplasm collection show a particular phenotype usually described by breeders as egusi seed type. The classification of these accessions has been confusing because there are literature references to the egusi type as belonging to either *Colocynthis citrullus* or to *C. lanatus* (Maynard 2001). Typically *C. citrullus* has been confused with *Citrullus colocynthis*, and as a result the egusi watermelon has sometimes been considered a common name for *C. colocynthis* (Maynard 2001). However, it is important to distinguish *C. lanatus* [= *C. citrullus* L.] having the egusi phenotype from *C. colocynthis* Schrad., a different *Citrullus* species (commonly called colocynth), which grows wild in warm and arid areas of Africa and Asia (Oyolu 1977b). The egusi watermelon is commonly known in Nigeria and the Congo as wild watermelon, egusi melon, or ibara.

The egusi watermelon is widely cultivated in Nigeria (Anuebunwa 2000; Ezeike and Otten 1989, 1991; Jolaoso et al. 1996), where the protein- and carbohydrate-rich seeds are used as a regular part of the diet. The egusi watermelon fruit is not edible because of its bitter, hard, white flesh, and the seeds are often called kernels (Oyolu 1977b).

The origin of the egusi phenotype is uncertain and the developmental genetics of this seed phenotype are unknown. Its seeds are coated by an adherent layer of tissue that may be the remnants of nucellar tissues. The tissues are visible only after the second to third week of seed development (Figure 1A) and can be removed at maturity for commercial use of the seeds (Figure 1B). Egusi seeds have been classified into different types according to the thickness of the seed coat and the flatness of the edges. They have also been divided into three groups based on oil extraction characteristics (Oyolu 1977a). The seeds usually have a white or cream color and can be of different sizes (Oyolu 1977b). The objective of this study was to determine the inheritance of the egusi seed phenotype in watermelon.

Materials and Methods

In the experiment we used two families developed from two crosses, Charleston Gray × PI 560006, and Calhoun Gray ×PI 490383w. PI 490383 is described as segregating for seed type and seed color in the USDA-ARS Germplasm Resources Information Network (GRIN) database, but we used a selection made from that accession that was uniform and homozygous for seed type and color (PI 490383w; entry NCG-529 of the North Carolina Watermelon Gene Mutant Collection, Department of Horticultural Science, North Carolina State University, Raleigh, NC). Both of the adapted parents had the normal seed type, while both of the PI accessions had egusi seed type. The PI accessions, as well as

Figure 1. Egusi seed type in PI 490383w: the seeds have a fleshy pericarp when fresh **(A)** and are similar to normal watermelon seeds after cleaning and drying **(B)**.

the adapted cultivars, were classified as *C. lanatus* var. *lanatus* (USDA-ARS 2002). We used Charleston Gray and Calhoun Gray as parents because they have disease resistance and high fruit yield and quality (Wehner 2002). The two cultivars have been grown extensively and have also been used in the development of many of the commercial cultivars used in the American market.

We developed six generations (P_aS_1, P_bS_1, F_1, F_2, BC_1P_a, BC_1P_b) in the greenhouse of the Department of Horticultural Science (North Carolina State University, Raleigh, NC) and in the greenhouse of the USDA-ARS (Griffin, GA). We could not develop reciprocal crosses because the wild germplasm used as the carrier of the egusi trait did not set fruit when pollinated with pollen from the adapted parent. High levels of sterility were also encountered in the cross Charleston Gray × PI 560006, which explains the few individuals tested in some generations of this cross. Our field test was run at the Horticultural Crops Research Station (Clinton, NC) in the summer of 2001 (Charleston Gray × PI 560006) and summer of 2002 and 2003 (Calhoun Gray × PI 490383w). The experiment had four sets in 2001, two sets in 2002, and one set in 2003, each set including all six generations of the same family. Seeds of the six generations were planted in rows in the following order: P_aS_1, P_bS_1, F_1, BC_1P_a, BC_1P_b, F_2. Rows were thinned to provide an even plant spacing and each plant marked with a numbered stake. One fruit was harvested from each plant separately and cut for evaluation and data collection.

Plants were classified into egusi and normal phenotypes depending on the presence/absence of the egusi fleshy tissues adherent to the seeds. We never observed any intermediate trait that could have made our evaluation uncertain. We discarded data recorded on three fruits (two F_1 and one BC_1P_a individuals in the cross Calhoun Gray × PI 490383w in 2002) because they were harvested on plots invaded by branches of segregating F_2 plants in the adjacent row and tracing of the vine was not reliably possible. We did not find any questionable phenotype in the same family in 2003.

We analyzed the data by family and then pooled over families after testing for chi-square homogeneity (Ostle and Malone 1988; Steel et al. 1997), performing segregation analysis and goodness-of-fit tests with the SAS statistical package (SAS Institute, Cary, NC) and the SASGene 1.2 program (Liu et al. 1997).

Results and Discussion

The homogeneity chi-square (with df = 1) for the F_2 and BC_1P_b data was 0.07 and 0.17, respectively. Therefore we analyzed the data pooled over families and the two families as a further source of replication of the experiment.

In the F_1 generation, all fruit in the two crosses had normal seed type, suggesting that the egusi seed type was inherited as a single recessive gene. The pooled F_2 individuals segregated at the ratio 398/144 (normal/egusi): the chi-square was 0.71 ($\alpha = 0.05$, df = 1), showing that the data were consistent with a 3:1 ratio. The *P* value for this chi-square also confirmed a good fit of the data to the predicted values ($P = .39$).

The BC_1P_a individuals (P_a being the carrier of the normal seed type) showed only the normal phenotype, confirming the 1:0 expected ratio. The BC_1P_b individuals (P_b being the carrier of the egusi seed type) segregated at a ratio 60/49 (normal/egusi) that statistically corresponded to a 1:1 ratio ($\chi^2_{(0.05,\ 1)} = 1.11$, $P = .29$). Data by family confirm the same results and are summarized in Table 1. Our results confirm the hypothesis that egusi seed from PI 490383w and PI 560006, with seeds having a fleshy pericarp, is controlled by a single recessive gene. We propose naming this new gene *Egusi seed*, with symbol *eg*, in conformance with gene nomenclature rules for Cucurbitaceae (Cucurbit Gene List Committee 1982).

Table 1. Single-locus goodness-of-fit-test for egusi seed type in watermelon[a]

Generation	Total	Normal[b]	Egusi[c]	Expected[d]	χ^2	df	*P*
Calhoun Gray × PI 490383w (NCG-529)							
P_a[e]	9	9	0				
P_b[f]	9	0	9				
F_1	44	44	0				
F_2	433	319	114	3:1	0.41	1	0.52
BC_1P_a	120	120	0				
BC_1P_b	84	59	48	1:1	1.13	1	0.28
Charleston Gray × PI 560006							
P_a[e]	12	12	0				
P_b[f]	4	0	4				
F_1	4	4	0				
F_2	109	79	30	3:1	0.37	1	0.54
BC_1P_a	18	18	0				
BC_1P_b	2	1	1	1:1	0.00	1	1.00
Pooled							
P_a[e]	21	21	0				
P_b[f]	13	0	13				
F_1	48	48	0				
F_2	542	398	144	3:1	0.71[g]	1	0.39
BC_1P_a	138	138	0				
BC_1P_b	109	60	49	1:1	1.11[g]	1	0.29

[a] Data are ratings from two families of *Citrullus lanatus* var. *lanatus*: Calhoun Gray × PI 490383w and Charleston Gray × PI 560006; data are presented by family and pooled over families.

[b] Normal was the standard watermelon seed type without fleshy tissues adherent to the seed.

[c] Egusi was the mutant watermelon seed type with fleshy tissues adherent to the seed.

[d] Expected was the hypothesized segregation ratio for single-gene inheritance for each segregating generation.

[e] P_a was the carrier of the dominant gene (normal phenotype).

[f] P_b was the carrier of the recessive gene (egusi phenotype).

[g] Homogeneity $\chi^2 = 3.84$ (df = 1).

Acknowledgments

The authors gratefully acknowledge Tammy L. Ellington for assistance with the field tests, and Dr. Janet M. Spears for help with seed morphology descriptions.

References

Abd el Hafez AA, Gaafer AK, and Allam AMM, 1981. Inheritance of flesh colour, seed coat cracks and total soluble solids in watermelon and their genetic relations. I. Quantitative traits. Acta Agron Acad Sci Hung 30:82–86.

Anuebunwa FO, 2000. A bio-economic on-farm evaluation of the use of sweet potato for complementary weed control in a yam/maize/egusi/cassava intercrop in pigeon pea hedgerows in the rain forest belt of Nigeria. Biol Agric Hort 18:95–102.

Cucurbit Gene List Committee, 1979. New genes for the Cucurbitaceae. Cucurbit Genet Coop Rep 2:49–53.

Cucurbit Gene List Committee, 1982. Update of cucurbit gene list and nomenclature rules. Cucurbit Genet Coop Rep 5:62–66.

Ezeike GOI and Otten L, 1989. Two-compartment model for drying unshelled egusi (melon) seeds. Am Soc Agric Eng 89:1–20.

Ezeike GOI and Otten L, 1991. Two-compartment model for drying unshelled melon (egusi) seeds. Can Agric Eng 33:73–78.

Henderson WR, 1991. Gene list for watermelon. Cucurbit Genet Coop Rep 14:129–138.

Henderson WR, 1992. Corrigenda to 1991 watermelon gene list (CGC 14:129–137). Cucurbit Genet Coop Rep 15:110.

Jolaoso MA, Ojeifo IM, and Aiyelaagbe IOO, 1996. Productivity of plantain (Musa AAB)-melon mixtures in southwestern Nigeria. Biol Agric Hort 13:335–340.

Kanda T, 1951. The inheritance of seed-coat colouring in the watermelon. Jpn J Genet 7:30–48.

Kang S, Cho C, Kim Y, Kang SC, Cho CH, and Kim YK, 2000. Inheritance of seed and seed coat characters in watermelon (*Citrullus lanatus* (Thunb.) Matsum. et. Nakai). J Korean Soc Hort Sci 41:471–474.

Liu JS, Wehner TC, and Donaghy SB, 1997. SASGENE: a SAS computer program for genetic analysis of gene segregation and linkage. J Hered 88:253–254.

Maynard DN (ed), 2001. Watermelons. Characteristics, production, and marketing. Alexandria, VA: ASHS Press.

Ostle B and Malone LC, 1988. Statistics in research, 4th ed. Ames, IA: Iowa State University Press.

Oyolu C, 1977a. Extraction rates and chemical composition of seed types in egusi (*Colocynthis citrullus* L.). Acta Hort 53:287–290.

Oyolu C, 1977b. A quantitative and qualitative study of seed types in egusi (*Colocynthis citrullus* L.). Tropic Sci 19:55–62.

Poole CF, Grimball PC, and Porter DR, 1941. Inheritance of seed characters in watermelon. J Agric Res US 63:433–456.

Porter DR, 1937. Inheritance of certain fruit and seed characters in watermelons. Hilgardia 10:489–509.

Rhodes B, and Dane F, 1999. Gene list for watermelon. Cucurbit Genet Coop Rep 22:61–77.

Sharma RR and Choudhury B, 1982. Studies on inheritance of fruit and seed characters in watermelon. Veg Sci 9:89–95.

Steel RGD, Torrie JH, and Dickey DA, 1997. Principles and procedures of statistics: a biometrical approach, 3rd ed. Boston, MA: WCB/McGraw-Hill.

Tanaka T, Wimol S, and Mizutani T, 1995. Inheritance of fruit shape and seed size of watermelon. J Jpn Soc Hort Sci 64:543–548.

USDA-ARS. Germplasm Resources Information Network – (GRIN) (visited Oct. 13, 2004) http://www.ars-grin.gov/.

Wehner TC, 2002. Vegetable cultivar descriptions for North America. List 26-2002. HortScience 37:15–78.

Zhang J, 1996. Inheritance of seed size from diverse crosses in watermelon. Cucurbit Genet Coop Rep 19:67–69.

Zhen Qing F and Jin Hua L, 1995. The study of genetics on the main commercial characteristics in seed-watermelon. Acta Hort 402:37–40.

Received May 13, 2003
Accepted November 30, 2003

Corresponding Editor: Reid Palmer