

Seed Characterization and Relationships between Seed and Cotyledon Properties in *Lagenaria* spp. Accessions

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Abstract. Bottle gourd [*Lagenaria siceraria* (Molina) Standl.] is widely produced in some Asian and African countries as a fresh vegetable as well as for seed consumption. A major use of bottle gourd is for rootstocks in grafted watermelon production. There are several centers where bottle gourd genetic resources are maintained, with the U.S. Department of Agriculture (USDA) germplasm collection being one of the most important. There is little published information on the relationship between seed morphology and the early establishment of seedlings in bottle gourd. The objective of this study was to determine seed characterization, morphology, and cotyledon shape in 163 *Lagenaria* spp. accessions and measure any relationship between seed and cotyledon characteristics. In USDA *Lagenaria* germplasm, it has been determined that the common character in seeds was medium in terms of seed size (53% of accessions), intermediate in seed surface lustre (39% of accessions), brown in seedcoat color (89% of accessions), thin and uniform in seed margin (35% of accessions), and tan in seed margin color (64% of accessions). According to the research results, seed weight ranged from 0.11 g (PI 500820) to 0.36 g (PI 675112), seed length from 13.17 mm (PI 500820) to 23.68 mm (PI 675112), and seed width from 5.86 (PI 500808) to 11.21 mm (PI 491274). Cotyledon length ranged from 5.46 cm (PI 368640) to 2.47 cm (PI 381850). The widest cotyledon was 3.00 cm (PI 534552), and the narrowest was 1.50 cm (PI 381831). Interesting correlations were observed for seed weight with seed length ($R^2 = 0.259$), and cotyledon length with cotyledon width ($R^2 = 0.547$).

Bottle gourd is one of the first plant species domesticated for human use (Clarke et al., 2006; Schlumbaum and Vandorpe, 2012). Bottle gourd fruits are consumed as a vegetable in some African and Asian countries. Immature fruits are eaten after being cooked in the same way as *Cucurbita* spp. Mature fruits are scraped clean, and the dried, hard rind is used in making bowls, containers, musical instruments, decorations, and even fishing floats. Shoots, tendrils, and leaves of bottle gourd plants are also consumed by cooking (Manandhar, 2002; Moerman,

1998). Some bottle gourd types are mainly grown for edible seeds (Achigan-Dako et al., 2008). *Lagenaria* seeds are good sources of lipids and proteins (Achu et al., 2005; Loukou et al., 2007). There is a considerable interest in bottle gourd seeds because of their high nutritional quality, mainly in terms of protein and oil content (Pradhan et al., 2013). *Lagenaria siceraria* can also be used as a rootstock for watermelon to control soil-borne diseases and to manage stress from low soil temperature, excessive water, and salinity stress. Watermelon grafting onto bottle gourd was first used in Korea and Japan in the late 1920s (Ashita, 1927), the gourds having a high compatibility rate with watermelon (Lee, 1994; Oda, 1995; Yetişir and Sari, 2003).

L. siceraria, commonly known as bottle gourd or white-flowered gourd, has high morphological diversity. In its primary and secondary centers of origin, bottle gourd shows great morphological and genetic variability (Given, 1987). Multiple research groups have studied fruit size and shape (Morimoto et al., 2005; Xu et al., 2014; Yetişir et al., 2008), fruit rind thickness, fruit length, fruit width (Harika et al., 2012; Koffi

et al., 2009; Morimoto et al., 2005), seed morphotypes (Morimoto et al., 2005; Yetişir et al., 2008), and other agromorphological characteristics (Achigan-Dako et al., 2008; Morimoto et al., 2005, 2006; Sivaraj and Pandravada, 2005; Xu et al., 2014; Yetişir et al., 2008). A substantial variation among bottle gourd landrace in seed dimensions and seed yield was also reported by Buthelezi et al. (2019). Bottle gourd plants grown in a trailing system produced more seed yield with high quality than the traditional growing system (Sharma et al., 2016).

The most fundamental steps in the conservation and use of plant genetic resources are ensuring efficiency and sustainability in plant production. Seed is the basic tool of sustainability in generatively propagated plant species. Therefore, gene banks have undertaken the task of long-term protection of seeds against the loss of possible genetic diversity in the future. The U.S. Department of Agriculture, Agricultural Research Service (USDA–ARS) has one of the most important genetic resource collections in the world in *Lagenaria* as well as in many plant species. According to our knowledge, the previous study on properties of *L. siceraria* seed is limited, and there is no study about seed characteristics in the U.S. Genebank. Therefore, the objective of this study was to determine morphological characterization of seeds of bottle gourd genetic resources in USDA–ARS, to measure the quantitative seed characteristics, and to determine the correlations between seed and some seedling traits at the early seedling stage in 163 *Lagenaria* spp. accessions. This study is also important in that bottle gourds have seed measurements made with the greatest number of genetic resources.

Materials and Methods

The USDA–ARS, Plant Genetic Resources and Conservation Unit in Griffin, GA (<http://www.ars-grin.gov>) has 235 PI accessions of *L. siceraria* (Levi et al., 2009). In this study, all bottle gourd accessions in the collection were requested, and seed measurements were made in 163 that germinated from 180 accessions received. The experimental materials represented four continents. The codes and collection locations of 163 bottle gourds accessions used in this study are shown in Table 1.

Each accession was classified for five important qualitative traits following the standards of the descriptor list published by the Bioversity International for Cucurbitaceae (2007) as seed size (small, medium, large), seed surface lustre (dull, intermediate, glossy), seedcoat color (seedcoat absent, white, tan, yellow, orange, brown, gray, black), seed margin [absent, (Thin and uniform, Th-U), Thin and irregular (Tn-I), Thick and uniform (Tk-U), Thick and irregular (Tk-I)], seed margin color (seed margin absent, white, tan, yellow, orange, brown, gray, black). Ten seeds of each accession were measured for the following morphological

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Table 1. Seed characterization, seed and cotyledon measurements for 163 *Lagenaria* accessions.

Access. code	Accession ID	Origin	Seed size	Seed surface lustre	Seed coat color	Seed margin ^z	Seed margin color	Seed wt (g)	Seed length (mm)	Seed width (mm)	Cotyledon length (cm)	Cotyledon width (cm)
1	Grif 970	China, Beijing Shi	Small	Intermediate	Tan	Tk-U	Tan	0.12	13.64	6.04	4.25	2.47
3	Grif 15945	United States, IA	Large	Glossy	Brown	Tn-U	Brown	0.21	16.20	6.93	4.77	2.53
4	PI 170463	Turkey	Large	Glossy	Brown	Tn-U	Brown	0.19	16.39	7.30	4.67	2.65
5	PI 181913	Syria	Large	Glossy	Brown	Tk-U	Brown	0.22	18.41	7.31	4.98	2.58
6	PI 194994	Ethiopia	Large	Intermediate	Brown	Tk-U	Brown	0.18	15.90	7.16	4.23	2.44
7	PI 195321	Guatemala	Large	Intermediate	Brown	Tn-U	Tan	0.20	16.88	7.81	4.93	2.62
8	PI 269505	Pakistan	Medium	Dull	Brown	Tk-U	Brown	0.17	14.37	7.30	3.65	2.05
9	PI 269506	Pakistan	Large	Glossy	Brown	Tk-U	Tan	0.17	15.78	6.44	3.37	1.87
11	PI 270456	Mexico, Ciudad de México	Large	Intermediate	Brown	Tk-U	Brown	0.20	16.73	7.30	4.70	2.25
14	PI 271356	India	Large	Dull	Tan	Tn-U	Brown	0.14	17.81	7.99	3.70	2.00
15	PI 271360	India	Large	Dull	Tan	Tn-U	Tan	0.19	16.86	7.50	4.27	2.49
16	PI 287533	Italy	Medium	Intermediate	Brown	Tn-U	Brown	0.17	14.98	7.27	4.10	2.45
17	PI 287534	Italy	Large	Intermediate	Brown	Tk-U	Brown	0.14	16.12	6.76	3.83	1.93
18	PI 288497	India	Large	Intermediate	Brown	Tk-U	Brown	0.15	15.82	7.48	4.30	2.50
20	PI 288499	India	Large	Intermediate	Brown	Tk-U	Brown	0.16	14.80	7.27	3.40	2.20
21	PI 288500	India	Medium	Intermediate	Brown	Tk-U	Brown	0.19	15.18	7.77	2.65	2.21
24	PI 358045	Former Serbia and Montenegro	Large	Glossy	Brown	Tn-U	Tan	0.19	16.87	6.98	3.48	1.95
25	PI 358046	Former Serbia and Montenegro	Large	Intermediate	Brown	Tk-U	Brown	0.16	16.40	6.80	4.57	2.48
26	PI 358048	Former Serbia and Montenegro	Large	Dull	Brown	Tn-U	Brown	0.21	16.46	7.73	4.75	2.40
27	PI 358049	Former Serbia and Montenegro	Medium	Intermediate	Brown	Tn-U	Brown	0.15	16.00	7.04	4.88	2.75
28	PI 358050	Former Serbia and Montenegro	Large	Glossy	Brown	Tn-U	Tan	0.20	16.30	8.61	5.22	2.78
29	PI 358051	Former Serbia and Montenegro	Large	Intermediate	Brown	Tn-U	Tan	0.20	17.24	8.73	5.03	2.61
30	PI 358052	Former Serbia and Montenegro	Large	Glossy	Brown	Tk-U	Tan	0.21	18.34	7.58	4.20	2.30
31	PI 358053	Former Serbia and Montenegro	Medium	Intermediate	Brown	Tn-U	Brown	0.18	14.89	7.17	5.20	2.63
32	PI 358059	Former Serbia and Montenegro	Large	Intermediate	Brown	Tn-U	Tan	0.17	18.01	8.08	4.59	2.53
33	PI 368635	Former Serbia and Montenegro	Medium	Intermediate	Brown	Tn-U	Brown	0.17	16.27	7.04	4.20	2.42
34	PI 368638	Former Serbia and Montenegro	Small	Intermediate	Tan	Tn-U	Tan	0.11	14.77	6.96	4.22	2.32
35	PI 368639	Former Serbia and Montenegro	Large	Dull	Tan	Tn-U	Tan	0.12	16.40	6.39	3.99	2.20
36	PI 368640	Former Serbia and Montenegro	Large	Intermediate	Brown	Tn-U	Brown	0.18	17.38	7.09	5.46	2.70
37	PI 370474	Former Serbia and Montenegro	Large	Dull	Brown	Tk-U	Brown	0.17	17.91	6.99	4.73	2.67
38	PI 381822	India, Rajasthan	Large	Glossy	Brown	Tk-U	Tan	0.19	16.41	6.72	4.20	2.39
39	PI 381823	India, Rajasthan	Large	Glossy	Brown	Tk-U	Tan	0.20	15.97	7.37	4.38	2.50
40	PI 381825	India, Rajasthan	Large	Glossy	Brown	Tk-U	Tan	0.19	15.87	7.09	3.86	2.02
41	PI 381826	India, Rajasthan	Medium	Intermediate	Brown	Tk-U	Tan	0.18	14.96	7.10	3.50	1.87
42	PI 381827	India, Rajasthan	Medium	Dull	Brown	Tk-U	Brown	0.14	13.99	6.82	4.17	2.59
43	PI 381828	India, Rajasthan	Medium	Intermediate	Brown	Tk-U	Tan	0.17	14.61	7.28	4.32	2.50
44	PI 381829	India, Rajasthan	Medium	Intermediate	Brown	Tk-U	Brown	0.20	16.62	6.82	3.97	2.10
45	PI 381830	India, Rajasthan	Medium	Glossy	Brown	Tk-U	Brown	0.16	15.28	6.37	3.69	2.17
46	PI 381831	India, Rajasthan	Medium	Glossy	Brown	Tk-U	Tan	0.19	15.07	7.21	2.75	1.50
47	PI 381832	India, Rajasthan	Large	Intermediate	Brown	Tk-U	Tan	0.22	17.13	7.26	3.60	2.08
48	PI 381834	India, Rajasthan	Large	Intermediate	Tan	Tk-U	Tan	0.19	15.61	7.37	4.19	2.64
49	PI 381835	India, Rajasthan	Large	Glossy	Brown	Tk-U	Brown	0.19	15.93	7.28	3.71	2.34
50	PI 381836	India, Rajasthan	Large	Intermediate	Brown	Tk-U	Tan	0.23	16.30	7.39	4.12	2.26
51	PI 381837	India, Rajasthan	Medium	Glossy	Brown	Tk-U	Brown	0.18	16.09	6.96	4.40	2.33
52	PI 381838	India, Rajasthan	Medium	Glossy	Brown	Tk-U	Tan	0.23	17.06	7.48	3.66	2.08
53	PI 381839	India, Rajasthan	Medium	Glossy	Brown	Tk-U	Gray	0.20	15.67	7.43	3.97	2.39
54	PI 381840	India, Rajasthan	Medium	Glossy	Brown	Tk-U	Brown	0.18	15.23	6.87	4.33	2.50
55	PI 381842	India, Rajasthan	Medium	Dull	Brown	Tk-U	Brown	0.19	15.00	7.54	3.70	2.35
56	PI 381843	India, Rajasthan	Medium	Glossy	Brown	Tk-U	Brown	0.23	16.68	7.56	3.70	2.60
57	PI 381844	India, Rajasthan	Medium	Glossy	Brown	Tn-U	Tan	0.19	15.59	7.28	4.13	2.60

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Table 1. (Continued) Seed characterization, seed and cotyledon measurements for 163 *Lagenaria* accessions.

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58	PI 381845	India, Rajasthan	Medium	Intermediate	Brown	Tn-U	Tan	0.18	15.88	6.84	2.62	1.83
59	PI 381846	India, Rajasthan	Medium	Intermediate	Brown	Tk-U	Tan	0.20	15.81	7.09	2.60	1.63
60	PI 381847	India, Rajasthan	Medium	Intermediate	Brown	Tk-U	Tan	0.21	16.48	7.51	3.50	1.98
61	PI 381848	India, Rajasthan	Medium	Intermediate	Tan	Tk-I	Tan	0.20	16.91	7.66	2.93	1.68
62	PI 381849	India, Rajasthan	Large	Dull	Tan	Tk-I	Brown	0.19	16.83	7.09	3.43	2.15
63	PI 381850	India, Rajasthan	Medium	Glossy	Brown	Tk-U	Tan	0.20	15.34	7.30	2.47	1.60
64	PI 381851	India, Rajasthan	Medium	Glossy	Tan	Tk-U	Brown	0.15	14.20	7.09	3.53	2.28
65	PI 381854	India, Rajasthan	Medium	Intermediate	Brown	Tk-U	Brown	0.19	16.79	7.09	3.55	2.03
72	PI 438844	United States, MD	Medium	Glossy	Brown	Tn-U	Brown	0.18	15.53	7.96	4.18	2.33
73	PI 438846	Mexico	Medium	Glossy	Brown	Tn-U	Brown	0.17	14.22	8.13	3.82	2.34
74	PI 442368	Mexico	Medium	Dull	Black	Tk-I	Gray	0.21	15.64	10.94	4.44	2.49
75	PI 458736	Argentina	Medium	Glossy	Black	Tn-U	Tan	0.22	16.59	9.66	4.33	2.62
76	PI 470260	Indonesia	Large	Glossy	Tan	Tk-U	Tan	0.23	17.23	8.30	3.50	2.30
77	PI 487482	Israel	Medium	Intermediate	Brown	Tn-U	Tan	0.16	15.87	8.71	4.50	2.39
78	PI 491252	Greece	Medium	Intermediate	Brown	Tn-U	Brown	0.19	17.01	8.56	5.02	2.48
79	PI 491266	Zimbabwe	Small	Glossy	Brown	Absent	Absent	0.17	15.53	7.51	3.88	2.63
80	PI 491267	Zimbabwe	Small	Dull	Brown	Tk-I	Tan	0.20	16.74	7.92	4.65	2.63
81	PI 491269	Zimbabwe	Small	Dull	Brown	Tk-I	Brown	0.20	17.28	8.60	4.59	2.50
82	PI 491270	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.20	17.52	7.94	4.24	2.59
83	PI 491272	Zimbabwe	Medium	Dull	Brown	Tn-I	Tan	0.23	17.29	8.52	4.54	2.80
84	PI 491273	Zimbabwe	Small	Dull	Brown	Tn-I	Tan	0.15	13.73	7.78	3.75	2.64
85	PI 491274	Zimbabwe	Large	Glossy	Brown	Tk-U	Brown	0.31	19.91	11.21	4.00	2.43
86	PI 491275	Zimbabwe	Small	Glossy	Brown	Tn-I	Tan	0.16	15.12	7.94	4.13	2.43
87	PI 491276	Zimbabwe	Medium	Intermediate	Brown	Tn-I	Brown	0.17	15.82	7.61	4.31	2.70
88	PI 491277	Zimbabwe	Small	Dull	Brown	Tn-I	Brown	0.16	15.36	7.37	4.10	2.52
89	PI 491278	Zimbabwe	Small	Dull	Brown	Tk-I	Brown	0.17	15.10	7.01	4.34	2.57
90	PI 491280	Zimbabwe	Small	Intermediate	Brown	Tn-I	Brown	0.18	15.23	7.58	3.77	2.43
91	PI 491281	Zimbabwe	Medium	Intermediate	Brown	Tn-I	Brown	0.21	15.70	8.38	3.80	2.30
92	PI 491282	Zimbabwe	Small	Glossy	Brown	Tn-I	Tan	0.19	16.10	7.72	3.98	2.38
93	PI 491283	Zimbabwe	Medium	Dull	Brown	Tk-U	Tan	0.22	18.04	8.24	4.81	2.85
94	PI 491286	Zimbabwe	Small	Glossy	Brown	Tn-I	Tan	0.16	14.81	7.73	4.08	2.35
95	PI 491287	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.17	15.54	8.22	4.05	2.43
96	PI 491288	Zimbabwe	Medium	Dull	Brown	Tn-I	Tan	0.18	16.23	8.00	4.08	2.29
97	PI 491289	Zimbabwe	Small	Intermediate	Brown	Tk-I	Tan	0.16	15.61	7.88	3.74	2.20
98	PI 491290	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.19	15.70	7.51	4.66	2.62
99	PI 491291	Zimbabwe	Medium	Intermediate	Brown	Tk-I	Tan	0.20	16.53	8.78	4.08	2.38
100	PI 491292	Zimbabwe	Medium	Dull	Brown	Tn-I	Tan	0.18	14.87	7.62	4.20	2.27
101	PI 491293	Zimbabwe	Medium	Dull	Brown	Tn-U	Brown	0.20	16.22	8.44	4.08	2.38
102	PI 491294	Zimbabwe	Medium	Dull	Brown	Tn-U	Brown	0.17	14.31	8.11	4.33	2.41
103	PI 491295	Zimbabwe	Medium	Glossy	Brown	Tk-U	Brown	0.19	16.18	8.69	4.26	2.51
104	PI 491296	Zimbabwe	Large	Dull	Brown	Tn-I	Brown	0.25	18.29	8.32	4.22	2.56
105	PI 491297	Zimbabwe	Medium	Intermediate	Brown	Tn-I	Brown	0.20	16.93	8.06	3.90	2.23
106	PI 491298	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.20	16.18	7.80	3.82	2.15
107	PI 491299	Zimbabwe	Small	Intermediate	Brown	Tn-U	Tan	0.15	13.88	7.62	3.44	2.24
108	PI 491300	Zimbabwe	Medium	Intermediate	Brown	Tn-I	Tan	0.19	16.32	7.94	4.04	2.45
109	PI 491302	Zimbabwe	Medium	Intermediate	Brown	Tn-I	Tan	0.18	16.13	7.90	4.58	2.57
110	PI 491303	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.21	15.99	9.02	4.70	2.56
111	PI 491304	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.17	15.88	7.81	3.78	2.36
112	PI 491305	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.18	16.21	7.59	3.70	2.25
113	PI 491306	Zimbabwe	Small	Dull	Brown	Tn-I	Tan	0.15	14.37	6.87	3.84	2.32
114	PI 491307	Zimbabwe	Medium	Dull	Brown	Tn-U	Tan	0.23	16.14	8.41	4.00	2.63

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Table 1. (Continued) Seed characterization, seed and cotyledon measurements for 163 *Lagenaria* accessions.

Access. code	Accession ID	Origin	Seed size	Seed surface luster	Seed coat color	Seed margin ²	Seed margin color	Seed wt (g)	Seed length (mm)	Seed width (mm)	Cotyledon length (cm)	Cotyledon width (cm)
115	PI 491308	Zimbabwe	Small	Dull	Brown	Tn-I	Tan	0.18	14.34	7.77	3.96	2.50
116	PI 491309	Zimbabwe	Medium	Intermediate	Brown	Tn-I	Tan	0.17	15.83	7.46	4.46	2.46
117	PI 491311	Zimbabwe	Small	Dull	Brown	Tn-I	Tan	0.16	15.78	7.21	3.80	2.37
118	PI 491312	Zimbabwe	Small	Dull	Brown	Tn-I	Tan	0.17	15.34	7.41	4.03	2.37
119	PI 491313	Zimbabwe	Medium	Glossy	Brown	Tn-I	Tan	0.21	16.64	9.80	3.57	2.27
120	PI 491314	Zimbabwe	Medium	Glossy	Brown	Tn-I	Tan	0.20	16.48	8.72	4.27	2.50
121	PI 491315	Zimbabwe	Small	Glossy	Brown	Tn-U	Tan	0.17	14.62	7.69	3.85	2.35
122	PI 491316	Zimbabwe	Small	Intermediate	Brown	Tn-U	Tan	0.17	15.87	7.90	4.14	2.40
123	PI 491317	Zimbabwe	Small	Glossy	Brown	Tn-U	Tan	0.17	14.37	8.16	4.38	2.60
124	PI 491318	Zimbabwe	Large	Glossy	Brown	Tn-U	Tan	0.25	18.09	9.67	4.65	2.68
125	PI 491319	Zimbabwe	Medium	Dull	Brown	Tn-U	Tan	0.20	16.57	9.04	4.61	2.56
126	PI 491320	Zimbabwe	Medium	Dull	Brown	Tn-U	Absent	0.16	16.08	7.62	2.80	2.15
127	PI 491321	Zimbabwe	Medium	Dull	Brown	Tn-U	Tan	0.20	16.28	8.99	4.34	2.40
128	PI 491322	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.20	16.14	8.64	4.18	2.30
129	PI 491323	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.21	17.06	9.01	4.07	2.23
130	PI 491324	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.21	16.53	7.78	3.98	2.53
131	PI 491325	Zimbabwe	Medium	Dull	Brown	Tk-U	Brown	0.21	16.97	8.40	4.47	2.60
132	PI 491326	Zimbabwe	Small	Glossy	Brown	Tn-I	Tan	0.16	14.41	8.58	3.94	2.54
133	PI 491329	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.17	16.14	8.38	4.10	2.40
134	PI 491330	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.20	17.33	9.71	4.24	2.58
135	PI 491331	Zimbabwe	Small	Dull	Brown	Tn-I	Tan	0.15	14.84	7.57	3.70	2.40
136	PI 491332	Zimbabwe	Small	Intermediate	Brown	Tn-U	Tan	0.18	15.07	8.06	3.92	2.46
137	PI 491333	Zimbabwe	Small	Dull	Brown	Tn-I	Tan	0.19	15.49	8.29	3.83	2.55
138	PI 491335	Zimbabwe	Small	Intermediate	Brown	Tn-I	Tan	0.17	15.26	7.61	4.23	2.60
139	PI 491336	Zimbabwe	Small	Dull	Brown	Tk-I	Tan	0.15	15.59	7.28	4.08	2.46
140	PI 491337	Zimbabwe	Large	Dull	Brown	Tk-I	Tan	0.25	19.50	8.81	4.87	2.82
141	PI 491339	Zimbabwe	Large	Dull	Brown	Tk-I	Tan	0.18	17.50	7.06	4.20	2.45
142	PI 491343	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.19	15.86	8.61	4.67	3.00
143	PI 491346	Zimbabwe	Large	Intermediate	Brown	Tn-U	Brown	0.22	17.56	8.81	4.20	2.32
144	PI 491348	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.15	15.12	8.13	4.27	2.63
145	PI 491349	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.17	15.89	8.43	4.46	2.63
146	PI 491350	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.19	16.60	8.36	4.40	2.44
147	PI 491351	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.16	15.81	7.23	4.17	2.53
148	PI 491352	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.20	16.53	9.07	4.47	2.54
149	PI 491353	Zimbabwe	Medium	Dull	Brown	Tk-I	Brown	0.17	14.78	7.93	4.33	2.74
150	PI 491354	Zimbabwe	Medium	Dull	Brown	Tn-U	Tan	0.17	15.90	8.04	3.80	2.45
151	PI 491355	Zimbabwe	Small	Intermediate	Brown	Tn-U	Tan	0.16	14.37	7.59	4.12	2.48
152	PI 491356	Zimbabwe	Medium	Dull	Brown	Tn-I	Tan	0.20	15.47	7.54	4.30	2.85
153	PI 491357	Zimbabwe	Small	Intermediate	Brown	Tn-I	Tan	0.16	15.99	7.21	3.73	2.28
154	PI 491358	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.18	16.76	8.07	4.24	2.44
155	PI 491358	Zimbabwe	Medium	Intermediate	Brown	Tn-I	Tan	0.17	15.74	8.66	4.39	2.42
156	PI 491358	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.15	15.13	7.47	4.24	2.50
157	PI 491360	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.22	17.53	8.50	4.54	2.56
158	PI 491361	Zimbabwe	Medium	Dull	Brown	Tk-I	Brown	0.19	15.70	8.48	4.36	2.50
160	PI 491363	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.18	16.02	7.74	4.33	2.63
161	PI 491365	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.20	16.08	7.61	4.74	2.58
162	PI 491366	Zimbabwe	Medium	Glossy	Brown	Tn-U	Tan	0.20	17.06	8.39	4.40	2.64
163	PI 491367	Zimbabwe	Medium	Intermediate	Brown	Tn-U	Tan	0.20	13.70	7.43	3.98	2.36
164	PI 494865	Zambia	Small	Intermediate	Brown	Absent	Absent	0.15	13.98	5.86	3.83	2.20
165	PI 500808	Zambia	Small	Intermediate	Brown	Absent	Absent	0.12	13.17	6.76	3.84	2.28
166	PI 500820	Zambia	Small	Dull	Brown	Tn-I	Tan	0.11	13.17	6.76	3.84	2.28
167	PI 500828	Zambia	Small	Glossy	Brown	Tn-I	Tan	0.16	14.23	7.89	4.18	2.47

(Continued on next page)

Table 1. (Continued) Seed characterization, seed and cotyledon measurements for 163 *Lagenaria* accessions.

Access. code	Accession ID	Origin	Seed size	Seed surface lustre	Seed coat color	Seed margin ²	Seed margin color	Seed wt (g)	Seed length (mm)	Seed width (mm)	Cotyledon length (cm)	Cotyledon width (cm)
168	PI 534552	Syria	Large	Intermediate	Tan	Tn-U	Tan	0.24	17.94	7.59	4.97	3.00
169	PI 534556	Syria	Large	Intermediate	Tan	Tn-U	Tan	0.19	16.48	7.29	4.92	2.91
170	PI 636137	India, Karnataka	Large	Dull	Tan	Tk-U	Tan	0.19	16.12	7.56	4.64	2.72
171	PI 639723	Unknown	Medium	Intermediate	Tan	Tn-U	Tan	0.15	17.14	7.16	4.62	2.57
172	PI 641946	India	Medium	Glossy	Brown	Tk-U	Tan	0.17	15.59	6.54	4.40	2.55
173	PI 642045	United States, GA	Large	Intermediate	Tan	Tn-I	Tan	0.19	18.13	7.39	5.17	2.53
174	PI 658555	India	Medium	Glossy	Tan	Tk-U	Tan	0.15	15.59	6.71	4.57	2.73
176	PI 666109	Syria	Small	Dull	Brown	Tk-I	Brown	0.15	15.16	7.12	4.97	2.72
177	PI 675112	United States, CA	Large	Glossy	Brown	Tk-I	Brown	0.36	23.68	10.11	5.43	2.73
179	PI 668365	Spain	Medium	Glossy	Brown	Tn-U	Brown	0.21	17.39	7.28	5.44	2.95
180	PI 668366	Spain	Medium	Intermediate	Brown	Tn-U	Tan	0.20	17.96	7.07	4.89	2.36
F ratio	-	-	-	-	-	-	-	34.17	10.58	5.65	14.83	11.79
Prob>f	-	-	-	-	-	-	-	0.0001	0.0001	0.0001	0.0001	0.0001
LSD _{0.05}	-	-	-	-	-	-	-	0.013	1.098	0.962	0.372	0.203
Min	-	-	-	-	-	-	-	0.11	13.17	5.86	2.47	1.50
Max	-	-	-	-	-	-	-	0.36	23.68	11.21	5.46	3.00
Mean	-	-	-	-	-	-	-	0.19	16.08	7.77	4.16	2.42

²Tn-U = Thin and Uniform; Tn-I = Thin and Irregular; Tk-U = Thick and Uniform; Tk-I = Thick and Irregular.

traits: seed weight, seed length, and seed width. Seed dimensions (seed length and width) and seed weight were determined with 10 replications. Seed length and width were measured using a digital caliper, while seed weight was measured using a digital scale. Single-seed weight was determined with 0.01 precision balance.

Seeds of 163 bottle gourd accessions were sown in 48-cell flats filled with a mixture of sand and peat in a 1:1 ratio. The flats were placed in a 37-m² environmentally controlled greenhouse at the North Carolina State University Phytotron after sowing. The greenhouse was set to maintain the air temperature at 26/22 ± 3.0 °C (day/night), 400 ppm CO₂, 50% relative humidity, and a 14-h photoperiod that was supplemented using sixteen 1000-W metal halide lamps. Plants were watered twice daily: in the morning, with a standard phytotron nutrient solution (Thomas et al., 2005), and in the afternoon, with reverse osmosis water. Seedlings were kept in the greenhouse until the first true leaf stage. Cotyledon length and width of nine plants per treatment combination were measured with a ruler at the stage where the cotyledons were parallel to the ground.

The experiment was a randomized complete block design with nine replications of nine plants each. Data were subjected to analysis of variance (ANOVA) to investigate the differences among accessions. TABULATE and ANOVA procedures of SAS v9.0 statistical software package were used. Mean separations of accessions were tested using Fisher's protected least significant difference at $\alpha = 0.05$. Also, the correlation coefficients between all investigated variables, and regressions between significantly related variables, were calculated.

Results

High variation was observed in all the seed characteristics. Seed size was small in 34 (21%) accessions, medium in 87 (53%), and large in 42 (26%) accessions. Seed surface lustre was observed as dull in 44 (27%) accessions and glossy in 55 (34%) accessions. Sixty-four (39%) accessions were classified intermediate for this character. Most of the accessions (145 accessions; 89%) had brown or tan (16 accessions; 10%) seedcoat color. Black seedcoat color was determined in only two (1%) accessions. While no seed margin was found in only four of 163 accessions, seed margin was observed in the other 159 accessions (57 accessions had thin and uniform seed margin, 43 accessions had thin and irregular, 37 accessions had thick and uniform, and 22 accessions had thick and irregular). Most of the seed margin color was tan (105 accessions; 64%) and brown (52 accessions; 32%). Seed margin color was gray in two (1%) accessions, and absent in four (3%) accessions without a seed margin. Other colors were not observed (Table 1).

Highly significant ($P > 0.0001$) differences were observed among the accessions for all measured seed and cotyledon

Table 2. Correlation analysis in *Lagenaria* seed weight, length, width and cotyledons length and width.

	Seed wt (g)	Seed length (mm)	Seed width (mm)	Cotyledon length (cm)	Cotyledon width (cm)
Seed weight (g)	1.000	0.509	0.343	0.148	0.100
Seed length (mm)		1.000	0.259	0.213	0.095
Seed width (mm)			1.000	0.119	0.137
Cotyledon length (cm)				1.000	0.740
Cotyledon width (cm)					1.000

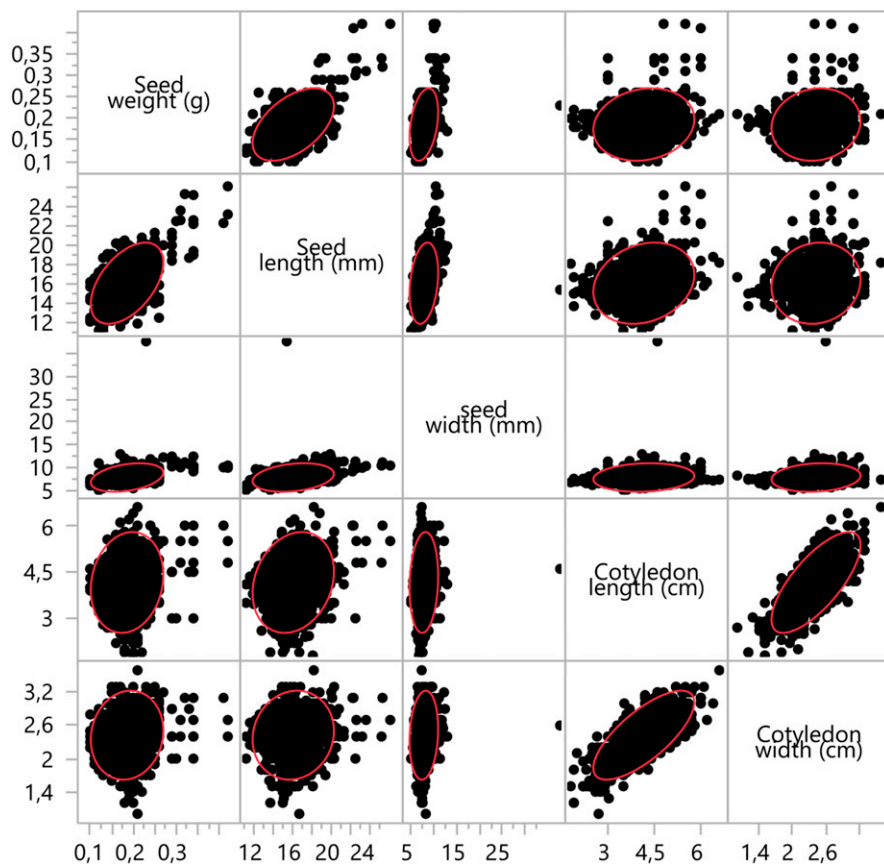


Fig. 1. Correlation diagrams in 163 *Lagenaria* accessions.

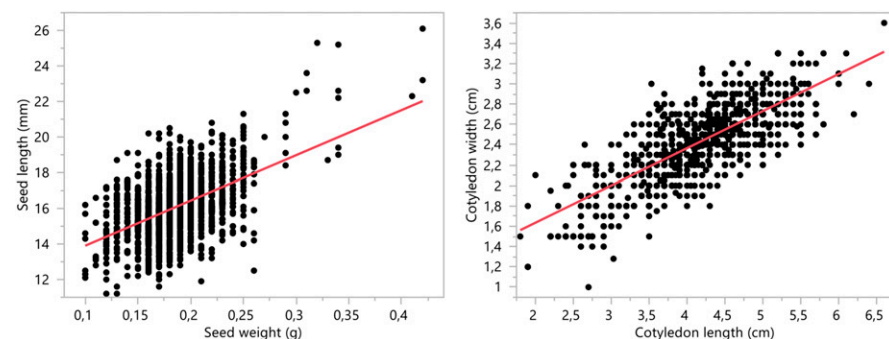


Fig. 2. Regression analysis between seed weight–seed length and cotyledon length–cotyledon width.

characteristics (Table 1). Seed weight ranged from 0.11 g to 0.36 g. The heaviest seeds were in PI 675112, PI 491274, and PI 491296; accessions with the lowest seed weight were in PI 500820, PI 368638, and PI 368639. In terms of seed length, the average value of 163 accessions was 16.08 mm, the maximum value was 23.68 mm, and the minimum value was 13.17 mm.

Accessions showed variations in seed length in a similar way to seed weight. The accessions with the longest seed length were PI 675112, PI 491274, and PI 491337, and the accessions with the shortest seed length were PI 500820, Grif 970, and PI 494865. For seed width, the average of 163 accessions was 7.77 mm, the maximum was 11.21 mm, and the minimum was 5.86 mm.



Fig. 3. Variation in seed morphology of USDA *Lagenaria* seed accessions.

The accessions with the largest cotyledon width were PI 491274, PI 442368, and PI 675112, and the accessions with the narrowest cotyledon widths were PI 500808, Grif 970, and PI 381830.

Cotyledon measurements after seed sowing for the accessions were also found to be statistically different. The longest cotyledons were in PI 368640, PI 668365, and PI 675112 accessions, while the shortest cotyledon accessions were in PI 381850, PI 381846, and PI 381845. Similarly, the accessions with the widest cotyledons were PI 534552, PI 491343, and PI 668365, while the narrowest were PI 381831, PI 381850, and PI 381846. The average cotyledon length for the 163 accessions was 4.16 cm, and cotyledon width was 2.42 cm. Cotyledon length varied from 2.47 cm to 5.46 cm, and cotyledon width varied from 1.50 to 3.00 cm.

The highest correlation value (74.0%) was determined between cotyledon length and width (Table 2). The correlation between seed weight and seed length was also highly significant (50.9%). The correlation between seed weight and seed width was 0.343, and the correlation between seed length and seed width was 0.259. The correlation value between seed length and cotyledon length was 0.213. Correlation values calculated among other parameters were below 20%; the smallest correlation was between seed length and cotyledon width. Correlations for all pairs of traits are shown in Fig. 1.

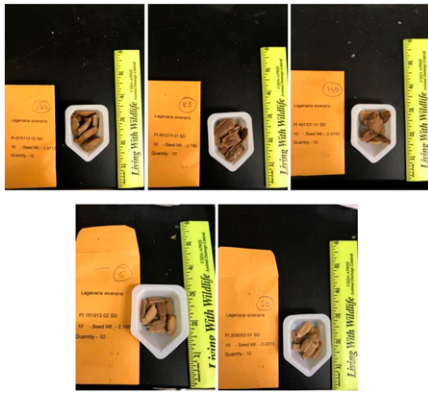


Fig. 4. The longest seeded *Lagenaria* accessions: 177 (PI 675112; 23.68 mm), 85 (PI 491274; 19.91 mm), 140 (PI 491337; 19.50 mm), 5 (PI 181913; 18.41 mm), and 30 (PI 358052; 18.34 mm).

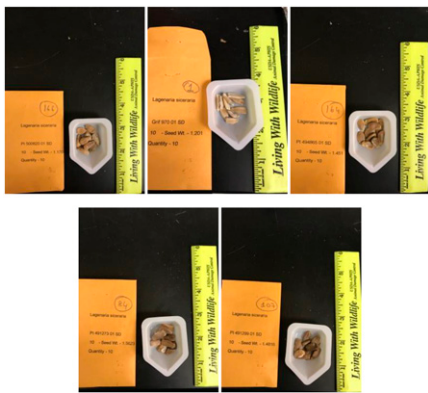


Fig. 5. The shortest seeded *Lagenaria* accessions: 166 (PI 500820; 13.17 mm), 1 (Grif 970; 13.64 mm), 164 (PI 494865; 13.70 mm), 84 (PI 491273; 13.73 mm), and 107 (PI 491299; 13.88 mm).

Regression analysis between important traits are shown in Fig. 2. The relationship between seed weight and seed length was found to be fully linear. In other words, accessions with heavier seed weight in all accessions had longer seeds. The cotyledon length and cotyledon width also showed a linear distribution, and all accessions were centered around this line. Seed diversity of USDA *Lagenaria* accessions is shown in Fig. 3, the accessions with the longest seed are presented in Fig. 4, while the shortest seeded accessions are presented in Fig. 5.

Discussion

Morphological characterization has been used by many scientists as a method of distinguishing differences among plant genetic resources (Solmaz et al., 2007). Seed characteristics are also important for characterization and identification of plants. In this study, which was carried out in 163 bottle gourd accessions of USDA (one of the important gene banks in the world), significant differences were found in terms of seed traits.

A study on South African *Lagenaria* germplasm on seed size, seed color, and seed margin (Buthelezi et al., 2019), and another study on Turkish germplasm on seed size, seed margin, and seed margin color (Yetişir et al., 2008) showed that *Lagenaria* had significant diversity in terms of seed characteristics.

Pradhan et al. (2013) reported various physical properties of bottle gourd seeds. Their results revealed that the average length, width, and thickness of seeds were 14.84, 7.44, and 3.34 mm, respectively. Buthelezi et al. (2019) collected a total of 12 landraces from the northern KwaZulu-Natal region of South Africa and determined seed length to be 14.0 to 23.0 mm, seed width 4.0 to 14.9 mm, and seed weight to be 0.12 to 0.22 g. Yetişir and Aydin (2019) reported the weight of 100 seeds to be 3.7 to 28.06 g, seed length to be 0.7 to 2.18 cm, and seed width to be 0.44 to 1.03 cm of 22 collected germplasms from different locations of Turkey. According to our research results, seed length ranged from 13.17 to 23.68 mm, seed width ranged from 5.86 to 11.21 mm, seed weight ranged from 0.11 to 0.36 mm—parallel with the results reported by those researchers. Seed size is considered one of the important seed morphology character in *Lagenaria* and other crops (Chimonyo and Modi, 2013; Kabalci and Sari, 2020; Solmaz and Sari, 2009; Yetişir et al., 2008). Morimoto et al. (2005) and Yetişir et al. (2008) were found to have significant variation and diversity in terms of the seed traits of bottle gourd landraces. Chimonyo and Modi (2013) found significant variation in their morphological examinations of bottle gourds collected from South Africa and Zimbabwe. Yetişir et al. (2005) collected a total of 182 *Lagenaria* genetic resources from Turkey, and Mashilo et al. (2016) collected a total of 36 landraces from South Africa and reported large seed and cotyledon size variation, depending on accession. In a study where Sharma et al. (2016) compared trailing and traditional growing methods, they found that single-seed weight was 0.16 g in the trailing method and 0.15 g in the traditional method. In our study, the seed weights ranged from 0.11 to 0.36 g, probably because we evaluated 163 *Lagenaria* accessions in our study, and Sharma et al. (2016) used just one cultivar, Pusa Naveen.

Some think that large seeds will be longer and the seedlings developed from those seeds will have larger cotyledons, but no relationship has been reported in the literature regarding the degree of relationship. In a study conducted on 'Galia'-type melon (Yetişir et al., 2004), this relationship was clearly revealed, and significant correlation was found between cotyledon and fruit shape. In our study, the most highly correlated traits were seed weight with seed length ($r = 0.51$), and cotyledon length with cotyledon width ($r = 0.74$).

Conclusions

As a result of this research, quantitative measurements were made on seeds of *Lage-*

naria spp. from the USDA germplasm collection, and seed sizes were determined. Bottle gourd accessions from four continents showed significant variation for seed biodiversity. In future studies, it may be useful to determine the relationship among traits involving seeds, cotyledons, leaves, and fruit in field and greenhouse conditions. In addition, relationships could be checked with resistance to diseases and pests.

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