



Developing a High-Throughput SNP-Based Marker Tightly Linked to Brittle-2 (Bt2) Gene in Super Sweet Corn

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Background

Sweetness is an economically important eating quality trait for sweet corn breeding. The various types of sweet corn are caused by single gene mutation [1]. The brittle2 and shrunken2 mutants accumulated 20% of starch found in the corn kernels [2, 3]. Due to mutation in ADP-glucose pyro phosphorylase reveals as a key regulatory enzyme to limit the rate of starch biosynthesis pathway [4-7]. The brittle2 (bt2) mutant is classified as class 1 super sweet corn [3,8,9], having 15-30% sugar of total carbohydrate content in the kernel, while normal corn contains about 3% sugar in the kernel [9,10]. Newly, the functional marker based on shrunken2 gene (sh2) was identified and developed [11]. This SNP marker designed within the coding region in exon 1 at position 154 of the coding sequence was clearly separated the sh2-based sweet corn from waxy corn, field corn, and the sweet corn that was based on the other genes, i.e., su1, se1, and bt2. However, there is no report of SNP markers associated with bt2 in sweet corn.

In this study, we scanned for SNPs from an Axiom® Maize 600k Genotyping Array from a collection of 6 sweet corn and 9 waxy corn recombination inbred lines (RILs). The SNPs were used to evaluate linkage-disequilibrium (LD) decays and association with bt2. Additionally, SNP-based markers tightly linked to bt2 were validated to find a marker associated with super sweet kernel trait that could aid in future super-sweet corn improvement programs.

Keywords: Maize; kernel Sweetness; Brittle 2; Single Nucleotide Polymorphism (SNP)

Results

A panel of 15 maize recombinant inbred lines (RILs) were used to obtain a set of 616,201 SNP variants on the bt2 using Axiom® Maize 600K Genotyping Array (Table S1). A panel of 331,945 variants were high-quality SNPs with minor-allele frequency (MAF) > 0.05 and missing rate < 10% and were included in subsequent analysis. Chromosome-wide LD among the 15 RILs, analyzed using PLINK software [12] revealed the 210 kb LD decay at a cut-off $r^2 = 0.2$ (Figure1). The bt2 (GRMZM2G068506) resides on chromosome 4 at position 58,954,361 to 58,960,521 bp (Maize Genome Database version2; <https://www.maizegdb.org>). We found that the AX-91607989 (C/T) SNP, located at 62,922 bp upstream of bt2,

was significantly associated with bt2-based sweet corn (Table S2). Among the 15 RILs, 2 lines controlled by bt2 had the T allele, while the others (sh2, su, se and wx) contained the C allele. A marker was developed using Mass ARRAY® platform and validated in a diverse panel of maize composing of inbred lines, hybrid lines, and RILs (Figure2; Table S3). This marker could clearly separate the bt2-based sweet corn from waxy corn, field corn, and other types of sweet corn mutants that was based on the other genes, i.e., su1, se1, and sh2 (Figure3; Table S4).

Finally, we extended the search for gene-based SNPs linked to bt2 on chromosome 4. One SNP was validated to differentiate

sweet corn from other corn types by possessing a *bt2* recessive allele (T). This SNP was located upstream of the *bt2* 62,922 base pair. We developed a marker tightly linked to the *bt2* and validated that the marker clearly distinguished the super sweetness corn from waxy corn, field corn, and the sweet corn controlled by other

mutants. The identified SNPs on the *bt2* could be used as selection marker for marker-assisted selection in maize-breeding programs. Moreover, the tightly linked markers identified in this study could effectively save breeders cost and valuable time from lengthy field trials, and aid in accelerating sweet corn breeding programs.

Table S1: List of 15 inbreds and recombinant inbred lines (RILs) used for genotyping in this study.

No	Sample name	Inbred/line/RIL	Genes/Traits	%Heterozygosity	Sucrose content (mg/g fresh weight)	Kernel phenotype	Classification
1	Pop. Bt	RIL	<i>bt2</i>	16.13	66.62	Shrunken, Opaque to tarnished	sweet corn
2	101LTSC10	RIL	<i>bt2sh2wx</i>	6.32	45.34	Shrunken, Opaque to tarnished	sweet corn
3	Pop. Su	RIL	<i>su</i>	24.02	26.33	Shrunken, Opaque to translucent	sweet corn
4	Pop. Se	RIL	<i>se</i>	23.87	27.93	Shrunken, Opaque to translucent	sweet corn
5	P4554/Su bu-1	RIL	<i>se</i>	6.727	30.99	Shrunken, Opaque to translucent	sweet corn
6	Pop Sh2	RIL	<i>sh2</i>	20.45	42.55	Shrunken, Opaque to translucent	sweet corn
7	209W	Inbred	<i>wx</i>	2.688	4.370274	Opaque	waxy corn
8	KV81	Inbred	<i>wx</i>	7.424	6.97	Opaque	waxy corn
9	KNM102	Inbred	<i>wx</i>	3.168	8.33	Opaque	waxy corn
10	Pop. Wx-4	RIL	<i>wx</i>	28.40	14.68	Opaque	waxy corn
11	Kv/Mon3-4	RIL	<i>wx</i>	17.94	4.60	Opaque	waxy corn
12	Waxy1-4	RIL	<i>wx</i>	23.27	3.84	Opaque	waxy corn
13	TB1	Inbred	<i>wx</i>	4.66	4.56	Opaque	waxy corn
14	TB3	Inbred	<i>wx</i>	6.11	5.19	Opaque	waxy corn
15	TC1	Inbred	<i>wx</i>	5.29	6.03	Opaque	waxy corn

Table S2: A single marker analysis of 66 SNP AXIOM ID validated on the 15 maize recombinant inbred lines (RILs).

No	SNP ID	Position	R2	PVE	P-value	
1	AX-90871489	58,820,203	ND	ND	ND	
2	AX-91607989	58,891,439	0.57	56.96	0.00	Bt_4_58891439
3	AX-90871486	58,891,467	ND	ND	ND	
4	AX-91607996	58,939,308	0.11	10.53	0.24	
5	AX-90871503	58,940,186	0.11	10.53	0.24	
6	AX-91468639	58,940,391	0.05	5.23	0.41	
7	AX-91218686	58,941,416	0.11	10.53	0.24	
8	AX-90871525	58,942,767	0.09	8.90	0.28	
9	AX-90871508	58,942,890	0.12	12.49	0.20	
10	AX-91607992	58,943,051	0.11	10.53	0.24	
11	AX-90871512	58,951,234	0.11	10.53	0.24	
12	AX-90871528	58,954,119	0.11	10.53	0.24	
13	AX-90871527	58,954,252	0.11	10.53	0.24	
14	AX-90871507	58,954,701	ND	ND	ND	<i>bt2</i> gene region
15	AX-91453443	58,954,964	0.06	5.50	0.40	<i>bt2</i> gene region
16	AX-91147434	58,956,243	0.04	4.04	0.47	<i>bt2</i> gene region
17	AX-91346465	58,956,443	0.04	4.04	0.47	<i>bt2</i> gene region
18	AX-91147438	58,956,482	ND	ND	ND	<i>bt2</i> gene region
19	AX-91346464	58,957,933	0.04	4.04	0.47	<i>bt2</i> gene region
20	AX-90541571	58,957,964	0.04	4.04	0.47	<i>bt2</i> gene region
21	AX-90871530	58,958,175	0.04	4.04	0.47	<i>bt2</i> gene region

22	AX-91607998	58,958,530	0.04	4.04	0.47	bt2 gene region
23	AX-91147436	58,958,602	0.05	4.79	0.43	bt2 gene region
24	AX-91218685	58,959,062	0.03	2.65	0.56	bt2 gene region
25	AX-91607991	58,959,079	0.04	4.04	0.47	bt2 gene region
26	AX-90871500	58,959,407	0.04	4.04	0.47	bt2 gene region
27	AX-90541570	58,960,129	0.20	19.60	0.10	bt2 gene region
28	AX-90871520	58,960,177	0.20	20.38	0.09	bt2 gene region
29	AX-90871524	58,960,248	0.20	20.38	0.09	bt2 gene region
30	AX-90612766	58,960,409	0.10	10.34	0.24	bt2 gene region
31	AX-90871522	58,960,517	0.20	20.38	0.09	bt2 gene region
32	AX-90871521	58,960,600	0.20	19.60	0.10	
33	AX-90871496	58,961,166	ND	ND	ND	
34	AX-90871514	58,970,014	0.09	8.90	0.28	
35	AX-91607995	58,976,468	ND	ND	ND	
36	AX-90871519	58,976,646	ND	ND	ND	
37	AX-91346461	58,976,890	0.05	5.23	0.41	
38	AX-91607994	58,978,082	ND	ND	ND	
39	AX-90871511	58,979,942	ND	ND	ND	
40	AX-90871504	58,999,000	0.1053	10.53	0.24	
41	AX-90871540	59,000,300	ND	ND	ND	
42	AX-90871557	59,024,109	0.11	10.53	0.24	
43	AX-90871542	59,025,496	ND	ND	ND	
44	AX-90871543	59,025,844	0.09	8.70	0.29	
45	AX-90871562	59,035,537	0.11	10.53	0.24	
46	AX-90541575	59,036,465	ND	ND	ND	
47	AX-90871555	59,039,844	0.11	10.53	0.24	
48	AX-90871553	59,050,082	0.11	10.53	0.24	
49	AX-91608009	59,058,669	0.11	10.53	0.24	
50	AX-90871547	59,059,189	0.11	10.53	0.24	
51	AX-91608006	59,061,103	0.11	10.53	0.24	
52	AX-90541577	59,065,795	0.11	10.53	0.24	
53	AX-90871537	59,067,946	0.09	8.70	0.29	
54	AX-90871551	59,068,100	0.07	7.18	0.33	
55	AX-91346470	59,069,101	0.07	7.16	0.33	
56	AX-90871532	59,069,135	0.11	10.53	0.24	
57	AX-91608011	59,074,500	0.11	10.53	0.24	
58	AX-91608019	59,075,063	0.11	10.53	0.24	
59	AX-91608015	59,081,312	0.11	10.53	0.24	
60	AX-90871554	59,093,942	0.11	10.53	0.24	
61	AX-90871591	59,143,160	0.11	10.53	0.24	
62	AX-90871593	59,143,220	0.11	10.53	0.24	
63	AX-90871592	59,143,421	0.11	10.53	0.24	
64	AX-90871590	59,149,915	0.05	4.54	0.45	
65	AX-90871582	59,153,544	0.11	10.53	0.24	
66	AX-90871568	59,153,930	0.11	10.53	0.24	

**orange; Bt_4_58891439, gray; SNP located on Bt2 gene; ND = not detected

Table S3: The Mass Array markers Bt2_4_58891439 designed based on SNP variant (C/T) in upstream bt2.

Markers	Chr	Position (V.2)	SNPs	Unextended primer (UEP) sequence (5'-->3')	UEP mass (Da)	Call 1	Mass 1 (Da)	Call 2	Mass 2 (Da)
Bt2_4_58891439	4	58,891,439	C/T	cATGCCCATGTCCACCA	5075.3	C	5322.5	T	5402.4

Table S4: List of 307 inbreds and recombinant inbred lines (RILs) used for marker validation in this study.

No	Sample name	Genes/Traits	Classification	Bt2_4_58891439
1	101L	<i>bt2wx</i>	sweet corn	T
2	no.10	<i>bt2sh2wx</i>	sweet corn	T
3	no.8	<i>bt2sh2wx</i>	sweet corn	T
4	101LTSC4	<i>bt2sh2wx</i>	sweet corn	T
5	D 2018	<i>se+</i>	sweet corn	C
6	Sweetness	<i>Synergistic (sh2se+)</i>	sweet corn	C
7	Montauk	<i>Synergistic (sh2se+)</i>	sweet corn	C
8	Allure	<i>Synergistic (sh2se')</i>	sweet corn	C
9	301/Mon3-1	<i>se</i>	sweet corn	C
10	TSC/H3-1	<i>sh2</i>	sweet corn	C
11	TSC/H3-7	<i>sh2</i>	sweet corn	C
12	TSC/Su75-1	<i>sh2</i>	sweet corn	C
13	TSC/Su75-2	<i>sh2</i>	sweet corn	C
14	TSC/Su75-3	<i>sh2</i>	sweet corn	C
15	TSC/Su75-4	<i>sh2</i>	sweet corn	C
16	TSC/Su75-5	<i>sh2</i>	sweet corn	C
17	TSC/Su75-6	<i>sh2</i>	sweet corn	C
18	TSC/Su75-7	<i>sh2</i>	sweet corn	C
19	TSC/Su75-8	<i>sh2</i>	sweet corn	C
20	TSC/Su75-12	<i>sh2</i>	sweet corn	C
21	TSC/Su75-13	<i>sh2</i>	sweet corn	C
22	KV81/SSW-4	<i>sh2</i>	sweet corn	C
23	s6248/x2573-1	<i>sh2</i>	sweet corn	C
24	ws21	<i>sh2</i>	sweet corn	C
25	ws26	<i>sh2</i>	sweet corn	C
26	ws160	<i>sh2</i>	sweet corn	C
27	ws181	<i>sh2</i>	sweet corn	C
28	ws186	<i>sh2</i>	sweet corn	C
29	ws243	<i>sh2</i>	sweet corn	C
30	Sweet5-5	<i>sh2</i>	sweet corn	C
31	Sweet5-6	<i>sh2</i>	sweet corn	C
32	Sweet5-7	<i>sh2</i>	sweet corn	C
33	Sweet7	<i>sh2</i>	sweet corn	C
34	KK2W	<i>sh2</i>	sweet corn	C
35	KK5Y	<i>sh2</i>	sweet corn	C
36	KK8W	<i>sh2</i>	sweet corn	C
37	KK8Y	<i>sh2</i>	sweet corn	C
38	SEY37	<i>sh2</i>	sweet corn	C
39	Hibrix3	<i>sh2</i>	sweet corn	C

40	Xtra-Tender 2171	<i>sh2</i>	sweet corn	C
41	TSC/SU75	<i>sh2</i>	sweet corn	C
42	CNSiA 17005	<i>sh2</i>	sweet corn	C
43	CNSiA 17016	<i>sh2</i>	sweet corn	C
44	CNSiA 17040	<i>sh2</i>	sweet corn	C
45	CNSiA 17074	<i>sh2</i>	sweet corn	C
46	CNSiA 17096	<i>sh2</i>	sweet corn	C
47	CNSiA 17130	<i>sh2</i>	sweet corn	C
48	CNSiA 17140	<i>sh2</i>	sweet corn	C
49	CNSiA 17164	<i>sh2</i>	sweet corn	C
50	CNSiA 17241	<i>sh2</i>	sweet corn	C
51	CNSiA 17242	<i>sh2</i>	sweet corn	C
52	CNSiA 17244	<i>sh2</i>	sweet corn	C
53	CNSiB 17075	<i>sh2</i>	sweet corn	C
54	CNSiB 17088	<i>sh2</i>	sweet corn	C
55	CNSiB 17173	<i>sh2</i>	sweet corn	C
56	CNSiB 17220	<i>sh2</i>	sweet corn	C
57	CNSiB 17248	<i>sh2</i>	sweet corn	C
58	CNSiE 17005	<i>sh2</i>	sweet corn	C
59	CNSiE 17006	<i>sh2</i>	sweet corn	C
60	CNSiE 17031	<i>sh2</i>	sweet corn	C
61	CNSiE 17052	<i>sh2</i>	sweet corn	C
62	CNSiE 17074	<i>sh2</i>	sweet corn	C
63	CNSiE 17085	<i>sh2</i>	sweet corn	C
64	CNSiE 17094	<i>sh2</i>	sweet corn	C
65	CNSiE 17100	<i>sh2</i>	sweet corn	C
66	CNSiE 17115	<i>sh2</i>	sweet corn	C
67	CNSiE 17119	<i>sh2</i>	sweet corn	C
68	CNSiE 17127	<i>sh2</i>	sweet corn	C
69	CNSiA 18040	<i>sh2</i>	sweet corn	C
70	CNSiA 18074	<i>sh2</i>	sweet corn	C
71	CNSiA 18103	<i>sh2</i>	sweet corn	C
72	CNSiA 18108	<i>sh2</i>	sweet corn	C
73	CNSiA 18112	<i>sh2</i>	sweet corn	C
74	CNSiB 18092	<i>sh2</i>	sweet corn	C
75	CNSiB 18069	<i>sh2</i>	sweet corn	C
76	CNSiB 18135	<i>sh2</i>	sweet corn	C
77	Clei 0838G	<i>sh2</i>	sweet corn	C
78	Clei 0856S	<i>sh2</i>	sweet corn	C
79	CNS 66	<i>sh2</i>	sweet corn	C
80	CNS 75	<i>sh2</i>	sweet corn	C
81	CNSiE 17028	<i>sh2</i>	sweet corn	C
82	CNSiE 17048	<i>sh2</i>	sweet corn	C
83	NSX/DKA/IDL-3	<i>wx</i>	waxy corn	C
84	216Y	<i>wx</i>	waxy corn	C
85	241W	<i>wx</i>	waxy corn	C
86	KV	<i>wx</i>	waxy corn	C

87	Y.18	wx	waxy corn	C
88	Orange	wx	waxy corn	C
89	H.NO.2	wx	waxy corn	C
90	TB1	wx	waxy corn	C
91	TW	wx	waxy corn	C
92	TB3	wx	waxy corn	C
93	F 4305	wx	waxy corn	C
94	M 80	wx	waxy corn	C
95	WTNGHB 003	wx	waxy corn	C
96	WPK 008	wx	waxy corn	C
97	WSJ 003	wx	waxy corn	C
98	WSL 001	wx	waxy corn	C
99	WPK 018	wx	waxy corn	C
100	WPK 034	wx	waxy corn	C
101	WPK 031	wx	waxy corn	C
102	WKRA 06	wx	waxy corn	C
103	KKCW 02	wx	waxy corn	C
104	PWHB 01	wx	waxy corn	C
105	YNB 01	wx	waxy corn	C
106	TWA 02	wx	waxy corn	C
107	WKA 005	wx	waxy corn	C
108	WALB 003	wx	waxy corn	C
109	WEWS 003	wx	waxy corn	C
110	WAGWX 001	wx	waxy corn	C
111	WKNN 016	wx	waxy corn	C
112	WKNN 004	wx	waxy corn	C
113	2A-4-1	wx	waxy corn	C
114	2A-4-2	wx	waxy corn	C
115	8A-3	wx	waxy corn	C
116	8A-4	wx	waxy corn	C
117	12A-1	wx	waxy corn	C
118	12A-2	wx	waxy corn	C
119	12C-15	wx	waxy corn	C
120	13A-1	wx	waxy corn	C
121	13A-2	wx	waxy corn	C
122	13A-5	wx	waxy corn	C
123	13A-8	wx	waxy corn	C
124	Y.18White-3	wx	waxy corn	C
125	Y.18White-6	wx	waxy corn	C
126	C.13-1White-1	wx	waxy corn	C
127	C.13-1White-3	wx	waxy corn	C

128	H-No.2Purple-2	wx	waxy corn	C
129	Waxy11-2	wx	waxy corn	C
130	Tein1-1	wx	waxy corn	C
131	Tein1-2	wx	waxy corn	C
132	Tein1-3	wx	waxy corn	C
133	Tein1-4	wx	waxy corn	C
134	Tein1-5	wx	waxy corn	C
135	Tein1-6	wx	waxy corn	C
136	Tein1-7	wx	waxy corn	C
137	Tein1-8	wx	waxy corn	C
138	Tein1-10	wx	waxy corn	C
139	Tein5-4	wx	waxy corn	C
140	Tein5-5	wx	waxy corn	C
141	Tein5-7	wx	waxy corn	C
142	PF/AB-2	wx	waxy corn	C
143	WSTS/IDL/KND	wx	waxy corn	C
144	TB/KND//IDL-1	wx	waxy corn	C
145	TB/KND//IDL-3	wx	waxy corn	C
146	DNRP-1	wx	waxy corn	C
147	DNRP-2	wx	waxy corn	C
148	DNRP-3	wx	waxy corn	C
149	DNRY-2	wx	waxy corn	C
150	DNRW-1	wx	waxy corn	C
151	DNRW-2	wx	waxy corn	C
152	DNRW-3	wx	waxy corn	C
153	DNRW-4	wx	waxy corn	C
154	DNRW-5	wx	waxy corn	C
155	DNRW-6	wx	waxy corn	C
156	DNRW-7	wx	waxy corn	C
157	DNRW-8	wx	waxy corn	C
158	DRP-1	wx	waxy corn	C
159	DRP-2	wx	waxy corn	C
160	DRP-3	wx	waxy corn	C
161	DRP-4	wx	waxy corn	C
162	DRP-5	wx	waxy corn	C
163	DRP-6	wx	waxy corn	C
164	DRP-7	wx	waxy corn	C
165	DRP-8	wx	waxy corn	C
166	DRP-9	wx	waxy corn	C
167	DRP-10	wx	waxy corn	C

168	DRP-11	wx	waxy corn	C
169	DRP-12	wx	waxy corn	C
170	DRP-13	wx	waxy corn	C
171	DRP-14	wx	waxy corn	C
172	DRP-15	wx	waxy corn	C
173	DRP-16	wx	waxy corn	C
174	DRP-17	wx	waxy corn	C
175	DRP-18	wx	waxy corn	C
176	DRP-19	wx	waxy corn	C
177	DRP-20	wx	waxy corn	C
178	DRY-1	wx	waxy corn	C
179	DRY-2	wx	waxy corn	C
180	DRY-3	wx	waxy corn	C
181	DRY-4	wx	waxy corn	C
182	DRY-5	wx	waxy corn	C
183	DRY-6	wx	waxy corn	C
184	DRY-7	wx	waxy corn	C
185	DRY-8	wx	waxy corn	C
186	DRY-9	wx	waxy corn	C
187	DRW-1	wx	waxy corn	C
188	DRW-2	wx	waxy corn	C
189	DRW-3	wx	waxy corn	C
190	DRW-4	wx	waxy corn	C
191	DRW-5	wx	waxy corn	C
192	DRW-6	wx	waxy corn	C
193	DRW-7	wx	waxy corn	C
194	DRW-8	wx	waxy corn	C
195	DRW-9	wx	waxy corn	C
196	DRW-10	wx	waxy corn	C
197	DRW-11	wx	waxy corn	C
198	DRW-12	wx	waxy corn	C
199	DRW-13	wx	waxy corn	C
200	DRW-14	wx	waxy corn	C
201	DRW-15	wx	waxy corn	C
202	DRW-16	wx	waxy corn	C
203	DRW-17	wx	waxy corn	C
204	DRW-18	wx	waxy corn	C
205	DRW-19	wx	waxy corn	C
206	DRW-20	wx	waxy corn	C
207	DRW-21	wx	waxy corn	C
208	DRW-22	wx	waxy corn	C
209	DRW-23	wx	waxy corn	C

210	DRW-24	wx	waxy corn	C
211	DRW-25	wx	waxy corn	C
212	DRW-26	wx	waxy corn	C
213	DRW-27	wx	waxy corn	C
214	DRW-28	wx	waxy corn	C
215	DRW-29	wx	waxy corn	C
216	LP-1	wx	waxy corn	C
217	LP-2	wx	waxy corn	C
218	LW-1	wx	waxy corn	C
219	LW-2	wx	waxy corn	C
220	LW-3	wx	waxy corn	C
221	RLP-1	wx	waxy corn	C
222	RLP-4	wx	waxy corn	C
223	RLW-1	wx	waxy corn	C
224	RLW-3	wx	waxy corn	C
225	RLW-4	wx	waxy corn	C
226	RLW-5	wx	waxy corn	C
227	RLW-8	wx	waxy corn	C
228	RLY-1	wx	waxy corn	C
229	RLY-2	wx	waxy corn	C
230	101LAB	wx	waxy corn	C
231	TW1	wx	waxy corn	C
232	Sein Gote To	wx	waxy corn	C
233	Kyout Nyin Pyaung	wx	waxy corn	C
234	Kayar Pyaung	wx	waxy corn	C
235	Thayat Thaimain	wx	waxy corn	C
236	Ngapyar Gyi	wx	waxy corn	C
237	Wxx 12 X 10	wx	waxy corn	C
238	Wxx 11X10 X Wxx 10X8	wx	waxy corn	C
239	Wxx 11X 10	wx	waxy corn	C
240	Inlay Ni	wx	waxy corn	C
241	KNM	wx	waxy corn	C
242	Tuxpeno-1	wx	waxy corn	C
243	2A-3	wx	waxy corn	C
244	12A-4	wx	waxy corn	C
245	12C-1	wx	waxy corn	C
246	12C-2	wx	waxy corn	C
247	12C-3	wx	waxy corn	C
248	12C-4	wx	waxy corn	C

249	12C-5	wx	waxy corn	C
250	12C-10	wx	waxy corn	C
251	12C-14	wx	waxy corn	C
252	99A-1	wx	waxy corn	C
253	99A-2	wx	waxy corn	C
254	99A-3	wx	waxy corn	C
255	99A-4	wx	waxy corn	C
256	99A-5	wx	waxy corn	C
257	Tein5-1	wx	waxy corn	C
258	Tein5-2	wx	waxy corn	C
259	S6248/TB/KND//PF-1	wx	waxy corn	C
260	S6248/TB/KND//PF-3	wx	waxy corn	C
261	DNRY-1	wx	waxy corn	C
262	RLP-2	wx	waxy corn	C
263	RLP-3	wx	waxy corn	C
264	RLW-7	wx	waxy corn	C
265	NS3	field corn	field corn	C
266	Ki56	field corn	field corn	C
267	Ki60	field corn	field corn	C
268	Ki48	field corn	field corn	C
269	Ki3	field corn	field corn	C
270	Ki44	field corn	field corn	C
271	Ki27	field corn	field corn	C
272	Ki26	field corn	field corn	C
273	ABI-18-004	field corn	field corn	C
274	ABI-18-015	field corn	field corn	C
275	ABI-18-037	field corn	field corn	C
276	ABI-18-039	field corn	field corn	C
277	ABI-18-002	field corn	field corn	C
278	ABI-15-015	field corn	field corn	C
279	ABI-17-011	field corn	field corn	C
280	Nei452004	field corn	field corn	C
281	Nei452026	field corn	field corn	C
282	Nei9008	field corn	field corn	C
283	Nei542010	field corn	field corn	C
284	Nei582016	field corn	field corn	C
285	Nei541017	field corn	field corn	C
286	Nei452006	field corn	field corn	C

287	Nei9202 (T)	field corn	field corn	C
288	Takfa 1	field corn	field corn	C
289	Nei452029	field corn	field corn	C
290	Takfa 3	field corn	field corn	C
291	Nei502007	field corn	field corn	C
292	Nei452029	field corn	field corn	C
293	Nei462013	field corn	field corn	C
294	Nei532005	field corn	field corn	C
295	Nei502002	field corn	field corn	C
296	Nei582042	field corn	field corn	C
297	Nei492024	field corn	field corn	C
298	Nei512013	field corn	field corn	C
299	Nei452008 (Takfa 1)	field corn	field corn	C
300	Nei452015 (Takfa 3)	field corn	field corn	C
301	Nei452006 (Takfa 4)	field corn	field corn	C
302	Nei462013 (Takfa 7)	field corn	field corn	C
303	Nei452009 (Takfa 5)	field corn	field corn	C
304	Ki56 SW16D-B5-1004-56	field corn	field corn	C
305	Ki56 SW16D-B5-1004-56-M	field corn	field corn	C
306	Ki56 SW16D-B5-1004-56-F	field corn	field corn	C
307	ABI-18-011	field corn	field corn	C

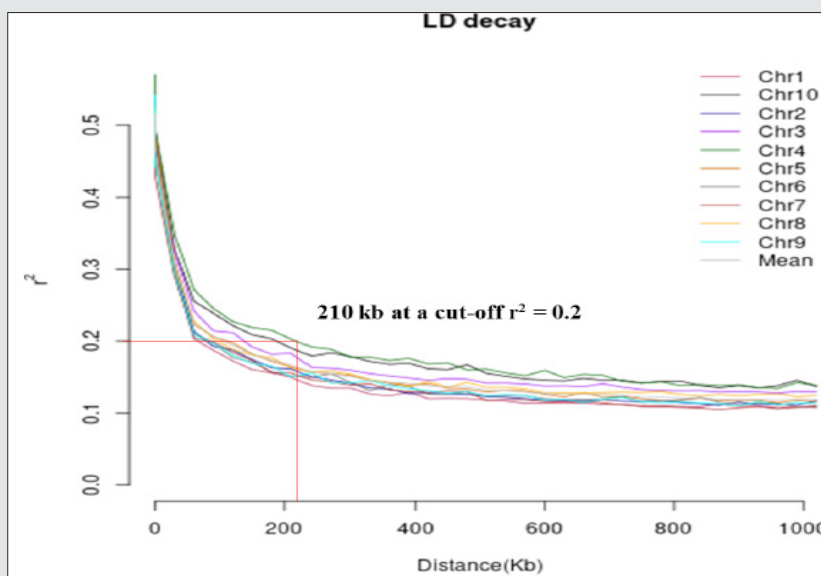


Figure1: Overall chromosome-wide linkage-disequilibrium (LD) decay estimated from single-nucleotide polymorphism (SNP) genotypes of 15 maize recombinant inbred lines. Each line plot represents a smoothed r^2 for all marker pairs on each chromosome depending on the distance between marker pairs.

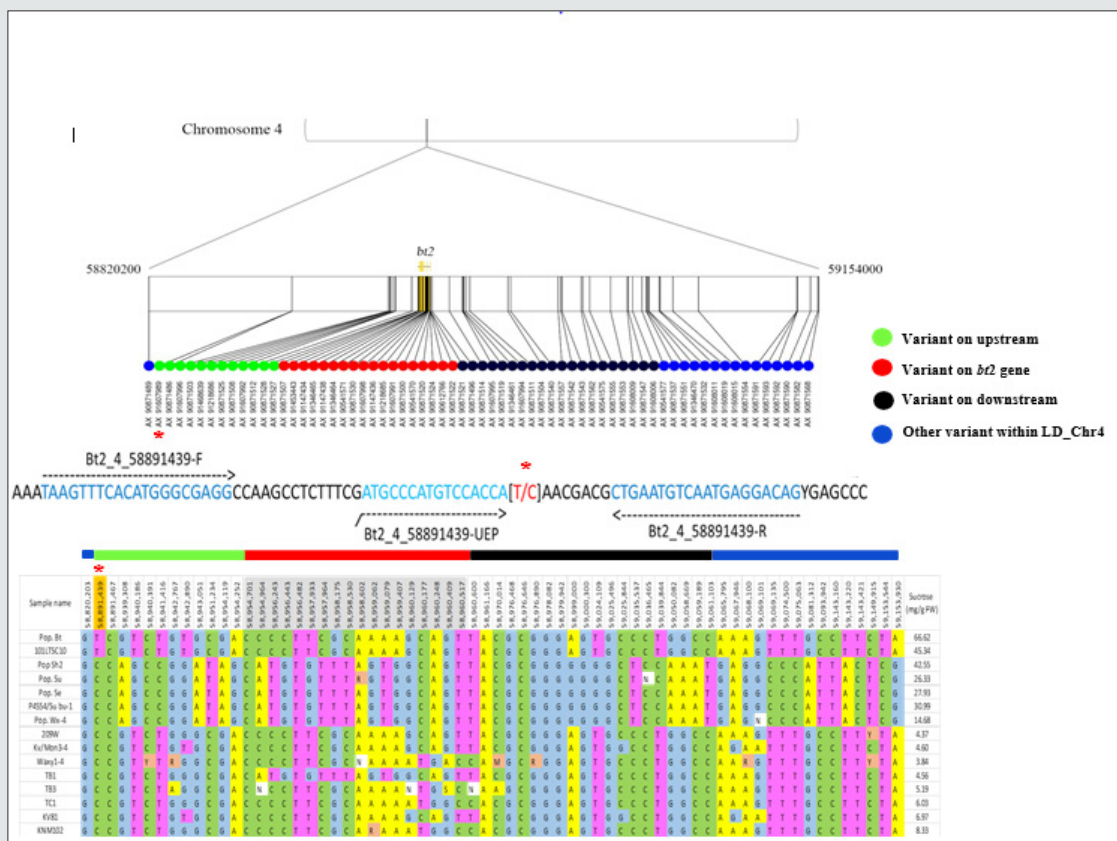


Figure 2: Physical location of the Bt2_4_58891439 SNP marker upstream of the brittle2 gene (bt2). Orange: Bt_4_58891439 SNP; gray: SNPs located in bt2 region. Sucrose content was analyzed from immature ears of each line at 24 days after pollination using the method described previously [13].

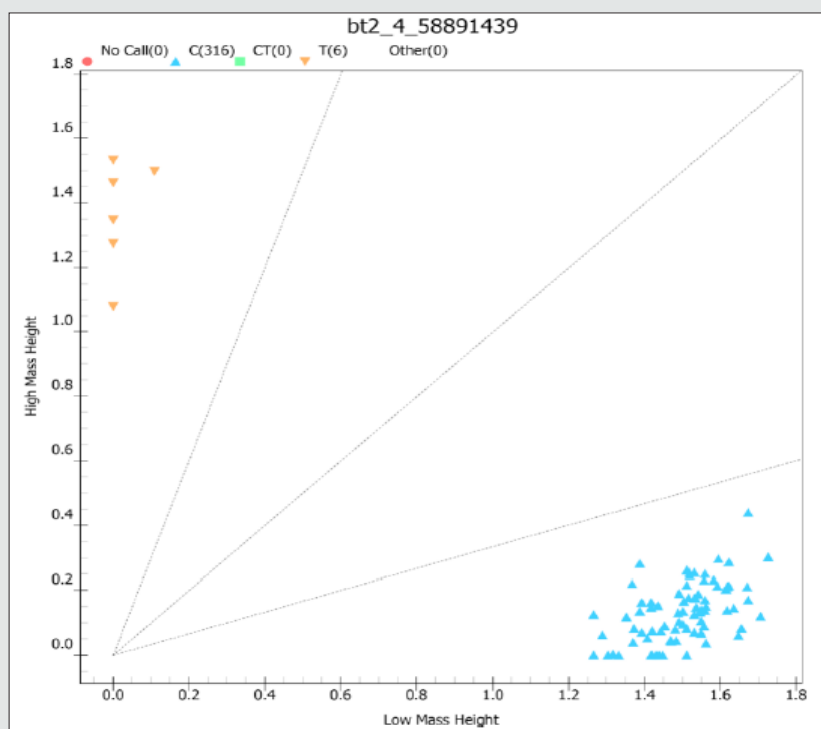


Figure 3: Allelic discrimination plot of SNP marker Bt2_4_58891439 validated in a panel of 322 maize lines as analyzed by Mass ARRAY® platform. Scatter dots with different colors showed clustering of homozygous genotype TT (yellow) and homozygous genotype CC (blue).

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Data Availability Statement

Data are contained within the article and the Supplementary Materials.

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Conflicts of Interest: The authors declare no conflict of interest.

References

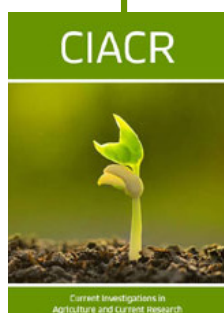
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