

Characterization of novel sorghum *brown midrib* mutants from an EMS-mutagenized population

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Table S1. Calibration statistics for NIRS[†] prediction of CP, NDF, ADF, ADL, Ash, and Total Carbon.

Trait	Math treatment [‡]	Calibration R ²	SEC	SECV	SEP	Validation R ²
			-----g kg ⁻¹ -----			
CP	2,8,6,1	0.989	0.228	0.303	0.517	0.982
NDF	4,10,10,1	0.939	1.371	1.563	2.231	0.882
ADF	3,10,10,1	0.955	0.852	1.107	1.164	0.931
ADL	3,5,5,1	0.882	0.373	0.606	0.577	0.805
Ash	3,10,10,1	0.963	0.476	0.662	0.449	0.937
Total Carbon	3,5,5,1	0.918	0.277	0.517	0.192	0.922

[†]Abbreviations: NIRS=near-infrared spectroscopy, CP=crude protein, NDF=neutral detergent fiber, ADF=acid detergent fiber, ADL=acid detergent lignin, SEC=standard error calibration, SECV=standard error cross validation, SEP=Standard Error of Prediction of validation set.

[‡]Math treatments used by NIRS software = derivative number, gap (nm over which derivative is calculated), smooth (number of points over which data is smoothed), second smooth (number of points).

Table S2. Midrib phenotype and genotypes of 26 non-*bmr* lines based on results of test crosses with *bmr2*, *bmr6*, and *bmr12* tester lines.

Line	Mutant Phenotype	Tester Line			Mutant locus
		AOK11 <i>bmr2</i>	AN603 ATx623 <i>bmr6</i>	AN604 ATx623 <i>bmr12</i>	
4	WT	WT	WT	WT	
25	WT	WT	WT	WT	
39	WT	-	WT	WT	
40	WT	WT	WT	WT	
41	<i>bmr</i>	WT	WT	WEAK	inconclusive
163	WT	WT	WT	WT	
247	WT	WT	WT	WT	
371	WT	WT	WT	WT	
372	WT	WT	WT	WT	
485	WT	WT	WT	WT	
492	WT	WT	WT	WT	
557	WT	WT	WT	WT	
666	WEAK	WT	WT	WT	inconclusive
706	WT	WT	WEAK	WT	inconclusive
924	WT	WT	WT	WT	
934	WT	WT	WT	WT	
1057	WT	WT	WT	WT	
1074	WT	WT	WT	WT	
1402	WT	WT	WT	WT	
1492	WT	WT	WT	WT	
1593	WT	WT	WT	WT	
1605	WT	WT	WT	WT	
1614	WT	WT	WT	WT	
1634	WT	WT	WT	WT	
1668	WT	WT	WT	WT	
1827	WT	WT	WT	WT	

The F₁ individuals, mutant lines, and check lines were visually classified as being *brown midrib* (*bmr*) or wild-type (WT) phenotype (See Materials and Methods). Based upon the test-crosses, loci and alleles were designated, which appear in the far right column.

Table S3. Evaluation of the agronomic traits for the mutant lines

Line	Stand Count (plants/m)			Time to 50% Anthesis (days after planting)			Height (cm)			Yield (T/ha)		
	Ismean	Lower	Upper	Ismean	Lower	Upper	Ismean	Lower	Upper	Ismean	Lower	Upper
BTx623 ^T	14.13	10.31	17.94	73.38	52.66	94.09	153.13	135.79	170.46	17.50	16.07	18.94
BTx623 ^N	16.63	12.95	20.30	72.75	52.28	93.22	148.13	128.63	167.62	17.26	14.19	20.34
RTx430	13.13	9.49	16.76	75.75	55.17	96.34	123.75	103.13	144.37	16.63	14.83	18.44
BWheatland	14.75	10.51	18.99	73.88	53.38	94.37	110.63	90.74	130.51	13.71	12.34	15.08
BTx623 <i>bmr6</i>	17.38	13.07	21.68	74.00	53.31	94.69	125.00	105.47	144.53	13.66	10.59	16.72
BTx623 <i>bmr12</i>	15.75	12.03	19.47	76.63	56.57	96.68	131.88	111.06	152.69	15.72	14.09	17.35
OK11 <i>bmr2</i>	15.13	11.48	18.77	78.00	57.39	98.61	120.00	101.98	138.02	12.02	10.09	13.94
<i>bmr2-2</i>	10.00	6.26	13.74	78.88	58.83	98.92	128.13	108.97	147.28	7.12	6.15	8.09
<i>bmr6-23</i>	15.13	11.24	19.01	78.50	58.65	98.35	96.25	79.52	112.98	5.23	4.25	6.21
<i>bmr6-31</i>	10.50	6.85	14.15	77.50	57.40	97.60	108.13	91.65	124.60	5.20	4.23	6.17
<i>bmr6-32</i>	12.88	9.25	16.50	78.00	57.60	98.40	115.63	94.34	136.91	9.76	8.68	10.83
<i>bmr6-45</i>	10.63	7.01	14.24	78.13	57.73	98.52	114.38	94.17	134.58	6.17	5.16	7.18
<i>bmr6-307</i>	6.25	2.47	10.03	81.63	61.29	101.96	112.50	94.06	130.94	3.48	1.99	4.96
<i>bmr6-741</i>	16.50	12.86	20.14	79.50	59.11	99.89	128.13	108.96	147.29	10.53	9.11	11.96
<i>bmr6-971</i>	14.88	11.02	18.73	79.00	58.69	99.31	125.00	105.80	144.20	11.72	10.47	12.98
<i>bmr6-1103</i>	13.13	9.36	16.89	79.13	59.06	99.19	91.88	71.92	111.83	8.26	6.88	9.64
<i>bmr6-1277</i>	10.75	6.40	15.10	76.88	56.53	97.22	104.38	83.20	125.55	7.95	6.66	9.24
<i>bmr12-30</i>	8.50	4.83	12.17	86.63	66.48	106.77	118.75	101.52	135.98	6.51	5.48	7.54
<i>bmr12-34</i>	12.88	8.96	16.79	82.83	62.43	103.23	133.13	115.58	150.67	8.45	7.40	9.49
<i>bmr12-35</i>	16.25	12.48	20.02	74.13	53.57	94.68	132.50	112.12	152.88	12.95	11.51	14.38
<i>bmr12-820</i>	11.21	7.49	14.93	89.37	71.06	107.67	126.83	107.39	146.26	4.83	3.53	6.13
<i>bmr29</i>	12.25	8.33	16.17	75.50	54.86	96.14	133.13	116.21	150.04	7.30	6.34	8.27
<i>bmr30</i>	5.25	0.57	9.93	84.00	64.28	103.72	128.75	111.38	146.12	4.77	3.53	6.00
<i>bmr31</i>	9.21	5.22	13.20	84.87	65.34	104.39	69.33	52.75	85.91	2.30	-0.43	5.03
<i>bmr32-1</i>	7.88	3.84	11.91	82.25	62.52	101.98	126.88	109.42	144.33	6.91	4.97	8.86
<i>bmr32-2</i>	11.88	8.15	15.60	76.13	55.43	96.82	115.00	97.51	132.49	8.26	7.26	9.27
<i>bmr32-3</i>	6.25	2.46	10.04	82.75	63.49	102.01	117.50	97.39	137.61	3.49	1.69	5.29
4	12.25	8.61	15.89	78.13	57.62	98.63	125.63	106.95	144.30	9.99	8.89	11.09
25	11.88	8.23	15.52	87.40	69.54	105.26	148.13	131.07	165.18	9.75	7.53	11.96
39	8.00	4.05	11.95	84.50	63.84	105.16	100.63	82.12	119.13	6.63	5.34	7.92
40	8.63	4.92	12.33	78.75	58.25	99.25	126.88	106.25	147.50	10.62	9.55	11.69
41	13.75	10.03	17.47	75.50	55.20	95.80	116.88	97.06	136.69	9.71	8.71	10.70
163	17.38	13.69	21.06	80.75	60.29	101.21	122.50	102.23	142.77	8.61	7.63	9.60
247	6.71	2.35	11.07	87.20	66.97	107.43	83.49	65.20	101.79	2.13	1.11	3.14
371	14.00	10.19	17.81	79.13	59.04	99.21	149.38	132.40	166.35	15.07	13.29	16.86
372	6.88	2.55	11.20	76.25	56.22	96.28	138.13	118.44	157.81	12.12	10.71	13.53
485	13.38	9.63	17.12	75.75	55.05	96.45	144.38	124.26	164.49	11.09	9.89	12.28
492	10.75	7.11	14.39	85.25	65.89	104.61	107.50	87.33	127.67	5.89	4.93	6.84
557	9.75	6.10	13.40	90.63	70.09	111.16	130.00	113.36	146.64	7.92	6.95	8.90
666	14.75	10.92	18.58	77.75	57.44	98.06	134.38	113.26	155.49	9.48	7.39	11.57

706	7.50	3.85	11.15	84.13	63.88	104.37	108.75	89.78	127.72	4.28	3.10	5.46
924	15.00	11.35	18.65	76.13	55.58	96.67	99.38	80.46	118.29	9.19	7.58	10.80
934	8.25	3.90	12.60	88.38	69.42	107.33	103.13	82.20	124.05	2.76	0.99	4.53
1057	10.38	6.72	14.03	75.38	55.08	95.67	119.38	99.14	139.61	6.38	5.43	7.33
1074	14.25	10.32	18.18	74.88	54.21	95.54	135.00	113.92	156.08	13.39	11.81	14.97
1402	9.75	5.81	13.69	82.38	62.62	102.13	117.50	96.89	138.11	6.00	4.50	7.51
1492	4.75	1.06	8.44	79.50	59.00	100.00	121.25	100.90	141.60	4.45	1.78	7.11
1593	13.50	9.01	17.99	87.71	68.09	107.32	90.63	74.61	106.64	3.81	2.80	4.81
1605	11.13	7.30	14.95	77.63	57.28	97.97	166.25	146.41	186.09	9.83	8.51	11.15
1614	11.00	7.18	14.82	77.13	56.64	97.61	128.75	109.01	148.49	8.37	7.17	9.58
1634	8.13	4.46	11.79	86.63	66.51	106.74	99.38	78.94	119.81	3.79	2.84	4.73
1668	11.13	7.51	14.74	79.38	59.43	99.32	113.13	96.02	130.23	7.17	6.14	8.20
1827	11.50	7.87	15.13	79.00	58.57	99.43	129.38	110.83	147.92	7.08	5.80	8.37

Measurement of these traits is as described in Materials and Methods. Yield is defined as the total above ground biomass. Least Squares Means (lsmean) for mutant and check lines were generated, and means of all variables were ranked from highest (Upper) to lowest (Lower). **Bold text** indicates values that were significantly different ($P \leq 0.20$) from the values of BTx623^T, the line used for mutagenesis. Additional details of the statistical analysis are described in Materials and Methods. BTx623^N is the line maintained by ARS in Lincoln, NE, which was included for comparison.

Table S4. NIRS prediction for NDF, ADF and ADL of the mutant lines

Line	NDF (%)			ADF (%)			ADL (%)		
	Ismean	Lower	Upper	Ismean	Lower	Upper	Ismean	Lower	Upper
BTx623 ^T	60.86	58.85	62.86	34.23	33.50	34.97	5.18	4.98	5.39
BTx623 ^N	61.48	59.48	63.49	34.52	33.76	35.28	5.34	5.13	5.54
RTx430	64.41	62.40	66.42	36.41	35.69	37.13	5.11	4.92	5.31
BWheatland	64.03	62.02	66.03	36.04	35.32	36.76	5.13	4.93	5.33
BTx623 <i>bmr6</i>	60.76	58.75	62.76	33.76	32.97	34.54	4.28	4.07	4.49
BTx623 <i>bmr12</i>	62.68	60.68	64.69	34.60	33.81	35.39	4.09	3.88	4.31
OK11 <i>bmr2</i>	64.79	62.78	66.79	35.53	34.79	36.27	4.05	3.85	4.25
<i>bmr2-2</i>	58.84	56.84	60.84	31.96	31.22	32.70	3.67	3.47	3.88
<i>bmr6-23</i>	60.84	58.83	62.85	32.82	32.13	33.51	4.59	4.40	4.77
<i>bmr6-31</i>	59.10	57.09	61.11	31.19	30.49	31.89	3.77	3.58	3.97
<i>bmr6-32</i>	61.02	59.02	63.02	33.05	32.31	33.78	3.82	3.62	4.02
<i>bmr6-45</i>	54.98	52.98	56.99	29.30	28.60	30.00	3.72	3.53	3.91
<i>bmr6-307</i>	58.15	56.14	60.15	29.13	28.35	29.91	4.30	4.09	4.51
<i>bmr6-741</i>	59.88	57.87	61.88	32.49	31.77	33.20	3.92	3.73	4.12
<i>bmr6-971</i>	60.14	58.14	62.15	32.59	31.84	33.35	3.97	3.77	4.18
<i>bmr6-1103</i>	59.44	57.43	61.44	32.30	31.54	33.06	3.74	3.54	3.95
<i>bmr6-1277</i>	68.66	66.66	70.67	38.56	37.87	39.26	5.08	4.89	5.27
<i>bmr12-30</i>	61.02	59.02	63.03	32.40	31.64	33.16	3.85	3.64	4.05
<i>bmr12-34</i>	61.52	59.52	63.53	33.25	32.47	34.03	3.75	3.53	3.96
<i>bmr12-35</i>	61.80	59.79	63.80	33.86	33.11	34.62	4.61	4.41	4.82
<i>bmr12-820</i>	58.46	56.44	60.49	31.12	30.25	31.98	3.37	3.13	3.60
<i>bmr29</i>	64.41	62.41	66.42	37.32	36.58	38.06	4.65	4.45	4.85
<i>bmr30</i>	61.29	59.28	63.30	32.31	31.51	33.12	4.50	4.28	4.72
<i>bmr31</i>	60.07	58.06	62.08	31.48	30.67	32.29	4.80	4.58	5.02
<i>bmr32-1</i>	62.34	60.33	64.34	33.52	32.78	34.26	4.02	3.82	4.22
<i>bmr32-2</i>	63.47	61.47	65.47	35.15	34.41	35.89	4.34	4.14	4.54
<i>bmr32-3</i>	59.89	57.89	61.90	31.37	30.63	32.10	4.53	4.33	4.74
4	64.05	62.05	66.06	35.76	35.01	36.52	5.51	5.30	5.71
25	56.90	54.90	58.91	30.60	29.79	31.41	3.64	3.42	3.86
39	61.21	59.20	63.22	32.82	32.01	33.62	4.99	4.77	5.21
40	62.45	60.44	64.45	34.33	33.57	35.09	5.47	5.26	5.68
41	60.85	58.84	62.85	33.14	32.38	33.90	5.09	4.89	5.30
163	62.53	60.53	64.54	34.63	33.87	35.38	4.77	4.56	4.98
247	61.51	59.50	63.53	32.73	31.90	33.57	5.20	4.97	5.43
371	64.94	62.93	66.94	36.61	35.86	37.37	5.41	5.21	5.62
372	60.40	58.39	62.40	32.72	31.96	33.48	5.00	4.79	5.20
485	59.26	57.26	61.27	32.88	32.15	33.62	4.47	4.27	4.68
492	58.94	56.93	60.95	31.68	30.87	32.48	4.29	4.07	4.51
557	59.61	57.61	61.62	31.87	31.11	32.63	4.78	4.57	4.98
666	65.34	63.33	67.34	36.09	35.37	36.81	4.10	3.90	4.30

706	59.88	57.87	61.88	30.63	29.89	31.37	5.58	5.38	5.78
924	66.00	64.00	68.01	37.53	36.79	38.27	5.84	5.64	6.04
934	62.82	60.81	64.82	33.45	32.73	34.16	5.07	4.87	5.26
1057	61.66	59.66	63.67	33.60	32.86	34.33	4.71	4.51	4.91
1074	59.80	57.80	61.81	32.92	32.16	33.68	4.75	4.54	4.96
1402	57.51	55.51	59.52	30.97	30.21	31.73	4.23	4.02	4.44
1492	60.98	58.97	62.99	32.48	31.67	33.28	4.93	4.71	5.16
1593	55.73	53.72	57.74	29.59	28.91	30.27	4.14	3.95	4.32
1605	57.65	55.64	59.66	32.27	31.59	32.95	5.02	4.84	5.21
1614	62.41	60.41	64.41	34.75	34.00	35.51	5.72	5.51	5.92
1634	57.59	55.59	59.59	28.78	28.03	29.54	4.80	4.59	5.01
1668	59.55	57.55	61.56	31.57	30.79	32.35	4.70	4.49	4.91
1827	56.55	54.55	58.55	30.66	29.92	31.39	4.05	3.85	4.25

Values for neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin (ADL) concentration were determined by NIRS prediction based on the calibration equation (Table S1). Additional details are found in the Materials and Methods Section. Least Squares Means (lsmean) for mutant and check lines were generated, and means of all variables were ranked from highest (Upper) to lowest (Lower). **Bold text** indicates values that were statistically significantly different ($P \leq 0.20$) from the values of BTx623^T, the line used for mutagenesis. Additional details of the statistical analysis are described in the Materials and Methods section. BTx623^N is the line maintained by ARS in Lincoln, NE, which was included for comparison.

Table S5. NIRS prediction for Total Ash, Crude Protein and Total Carbon of the *bmr* mutant lines.

Line	Ash (%)			Crude Protein (%)			Total Carbon (%)		
	lsmean	Lower	Upper	lsmean	Lower	Upper	lsmean	Lower	Upper
BTx623 ^T	11.60	11.13	12.06	5.46	4.19	6.73	43.28	43.09	43.47
BTx623 ^N	11.72	11.25	12.20	5.76	4.50	7.02	43.29	43.09	43.48
RTx430	10.94	10.49	11.39	6.86	5.58	8.14	44.03	43.84	44.21
BWheatland	12.43	11.97	12.88	7.21	5.94	8.49	43.06	42.87	43.24
BTx623 <i>bmr6</i>	14.52	14.03	15.01	6.10	4.85	7.36	41.85	41.65	42.05
BTx623 <i>bmr12</i>	12.12	11.63	12.62	6.95	5.70	8.21	43.22	43.02	43.42
OK11 <i>bmr2</i>	12.59	12.13	13.06	6.66	5.39	7.93	42.82	42.63	43.01
<i>bmr2-2</i>	11.14	10.68	11.61	5.30	4.03	6.56	43.31	43.12	43.50
<i>bmr6-23</i>	12.28	11.84	12.71	7.19	5.90	8.49	43.03	42.85	43.21
<i>bmr6-31</i>	11.33	10.89	11.77	7.59	6.31	8.88	43.19	43.01	43.37
<i>bmr6-32</i>	10.85	10.38	11.31	6.98	5.71	8.25	43.63	43.45	43.82
<i>bmr6-45</i>	10.59	10.15	11.03	5.90	4.62	7.19	43.24	43.06	43.42
<i>bmr6-307</i>	13.26	12.77	13.75	8.48	7.22	9.74	42.48	42.28	42.68
<i>bmr6-741</i>	11.54	11.09	11.99	6.03	4.75	7.30	43.12	42.93	43.31
<i>bmr6-971</i>	11.91	11.43	12.39	6.32	5.06	7.58	43.00	42.80	43.19
<i>bmr6-1103</i>	11.66	11.19	12.14	6.21	4.95	7.47	43.04	42.85	43.24
<i>bmr6-1277</i>	13.33	12.89	13.77	7.03	5.74	8.31	43.17	42.98	43.35
<i>bmr12-30</i>	10.78	10.30	11.26	8.04	6.78	9.30	43.71	43.51	43.90
<i>bmr12-34</i>	9.76	9.27	10.25	6.58	5.32	7.83	44.10	43.90	44.30
<i>bmr12-35</i>	10.26	9.78	10.74	5.22	3.96	6.48	43.94	43.75	44.13
<i>bmr12-820</i>	9.85	9.31	10.39	7.57	6.32	8.81	43.68	43.46	43.89
<i>bmr29</i>	10.81	10.34	11.27	6.21	4.95	7.48	43.83	43.64	44.02
<i>bmr30</i>	10.53	10.02	11.03	7.63	6.38	8.88	43.84	43.64	44.04
<i>bmr31</i>	11.22	10.72	11.72	9.72	8.47	10.98	43.27	43.07	43.47
<i>bmr32-1</i>	7.92	7.45	8.38	5.22	3.95	6.50	44.65	44.46	44.84
<i>bmr32-2</i>	9.86	9.39	10.32	5.89	4.62	7.16	44.09	43.90	44.28
<i>bmr32-3</i>	10.43	9.96	10.89	8.23	6.96	9.49	43.77	43.58	43.96
4	11.50	11.02	11.98	7.20	5.94	8.46	43.47	43.28	43.67
25	7.41	6.90	7.91	4.97	3.72	6.22	44.48	44.28	44.68
39	10.49	9.98	10.99	7.83	6.58	9.08	43.94	43.73	44.14
40	12.23	11.75	12.70	6.93	5.67	8.19	43.28	43.08	43.47
41	10.80	10.32	11.27	6.11	4.86	7.37	43.47	43.27	43.66
163	10.18	9.70	10.66	6.03	4.77	7.29	43.99	43.80	44.18
247	12.14	11.62	12.66	9.94	8.69	11.19	43.39	43.18	43.60
371	11.12	10.64	11.59	5.00	3.74	6.26	43.59	43.40	43.78
372	11.36	10.89	11.84	6.35	5.09	7.61	43.40	43.21	43.59
485	10.14	9.67	10.60	4.47	3.20	5.74	43.49	43.31	43.68
492	9.31	8.81	9.82	6.60	5.35	7.85	44.14	43.94	44.34

557	10.87	10.40	11.35	7.48	6.22	8.74	43.48	43.28	43.67
666	8.29	7.84	8.75	4.95	3.67	6.23	44.87	44.68	45.05
706	11.07	10.61	11.54	7.68	6.42	8.95	43.20	43.01	43.39
924	12.18	11.72	12.65	7.27	6.00	8.54	43.70	43.51	43.89
934	11.70	11.25	12.15	9.04	7.77	10.32	43.46	43.27	43.64
1057	11.63	11.16	12.09	7.03	5.77	8.30	43.13	42.94	43.32
1074	11.24	10.77	11.72	5.90	4.64	7.16	43.20	43.01	43.39
1402	9.65	9.17	10.13	6.31	5.05	7.57	43.66	43.47	43.86
1492	10.67	10.16	11.17	6.96	5.71	8.21	43.63	43.43	43.84
1593	10.31	9.88	10.74	9.06	7.76	10.35	43.60	43.42	43.78
1605	9.82	9.39	10.25	6.13	4.83	7.42	43.85	43.67	44.03
1614	12.12	11.65	12.60	6.80	5.54	8.06	43.14	42.94	43.33
1634	10.53	10.05	11.00	7.41	6.15	8.67	43.53	43.34	43.73
1668	10.24	9.75	10.73	7.24	5.98	8.49	43.66	43.46	43.85
1827	9.63	9.16	10.09	5.14	3.87	6.41	43.73	43.54	43.92

Values for ash, crude protein and total carbon were determined by NIRS prediction based on the calibration equation (Table S1). Additional details are found in the Materials and Methods Section. Least Squares Means (lsmean) for mutant and check lines were generated, and means of all variables were ranked from highest (Upper) to lowest (Lower). **Bold text** indicates values that were statistically significantly different ($P \leq 0.20$) from the values of BTx623^T, the line used for mutagenesis. Additional details of the statistical analysis are described in the Materials and Methods section. BTx623^N is the line maintained by ARS in Lincoln, NE, which was included for comparison.

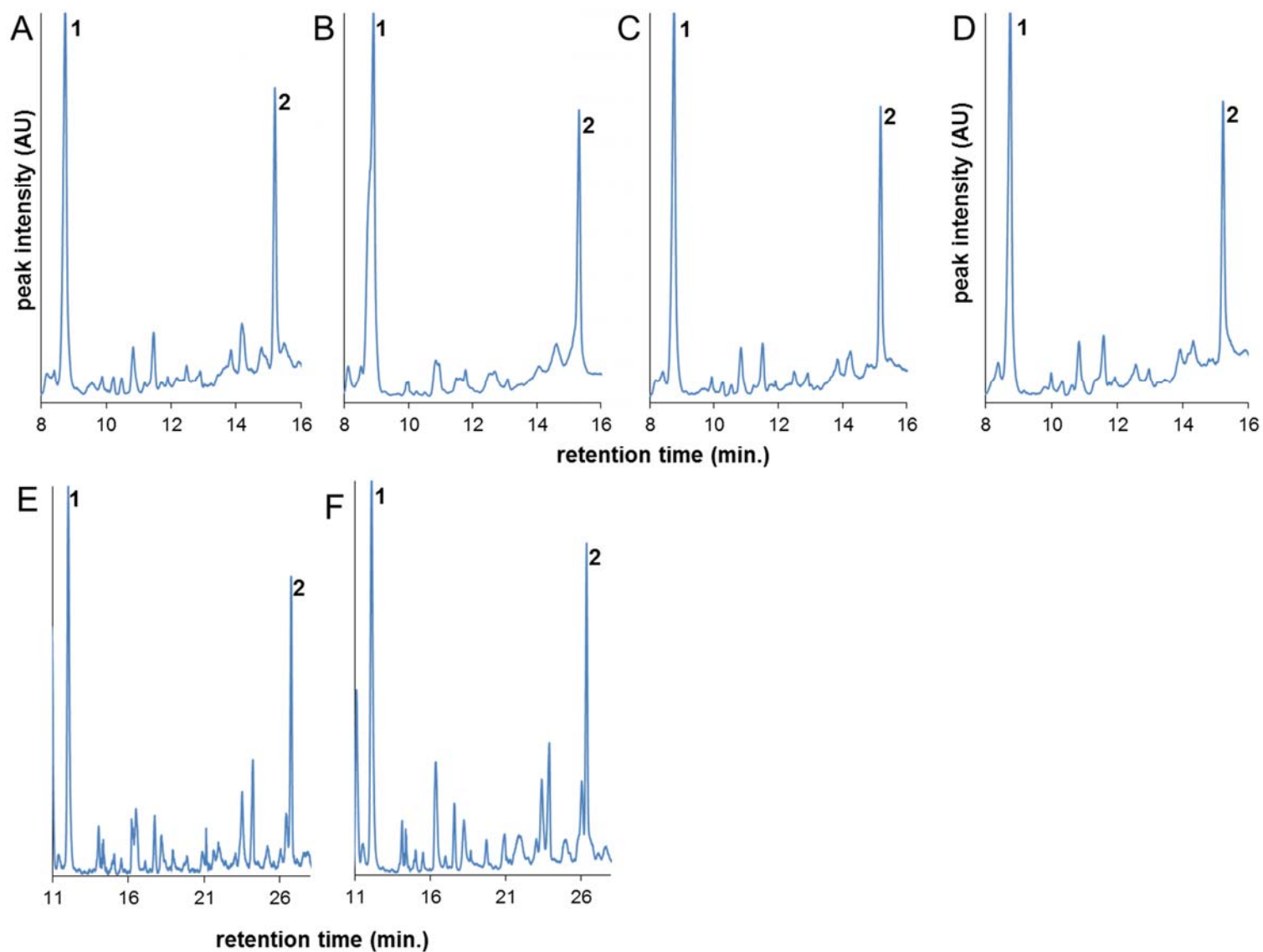


Figure S1 Partial pyrograms obtained with stover from **A.** BTx623, **B.** *bmr30*, **C.** *bmr31*, **D.** *bmr32-1*, **E.** BTx623, and **F.** *bmr29*. Peaks **1** and **2** represent 2-methoxy-4-methyl phenol (m/z 138, 123; derived from guaiacyl residues) and 2,6-dimethoxy-4-methyl phenol (m/z 168, 153; derived from syringyl residues). The difference in retention times between A-D and E, F reflects the slower ramp rate during gas chromatography of the latter two samples, to improve resolution.