MOLECULAR ECOLOGY

Supplemental Information for:

Multiple evolutionary origins or widespread gene flow of herbicide resistance in Bromus tectorum? Insights into genetic variation and population structure

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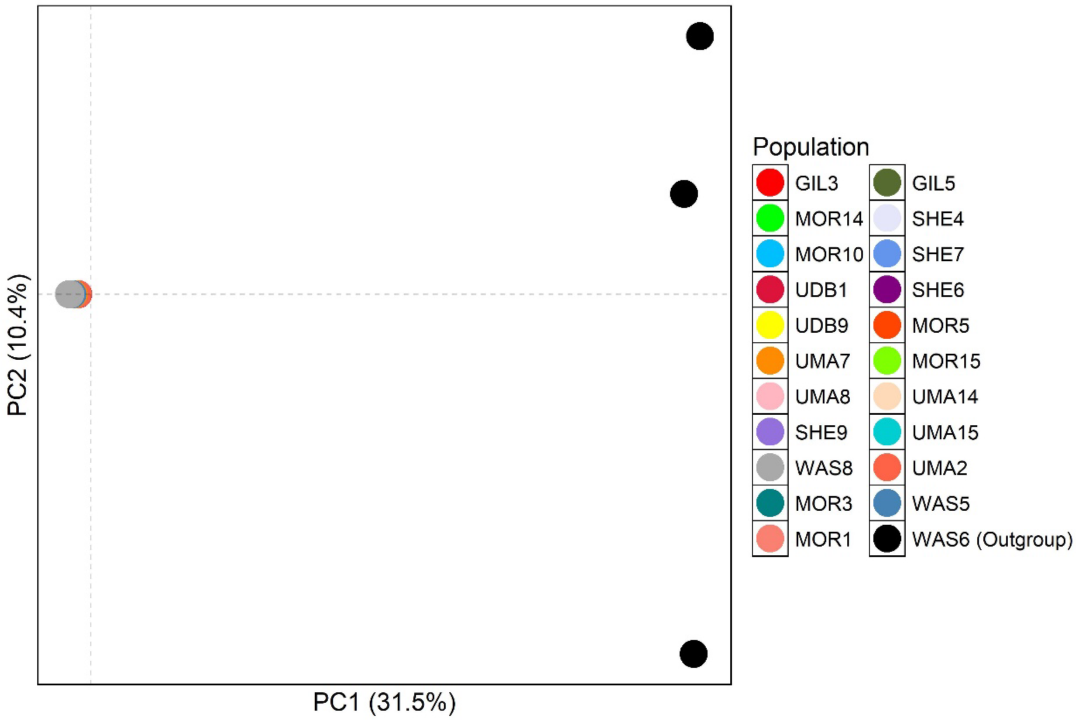
(a)



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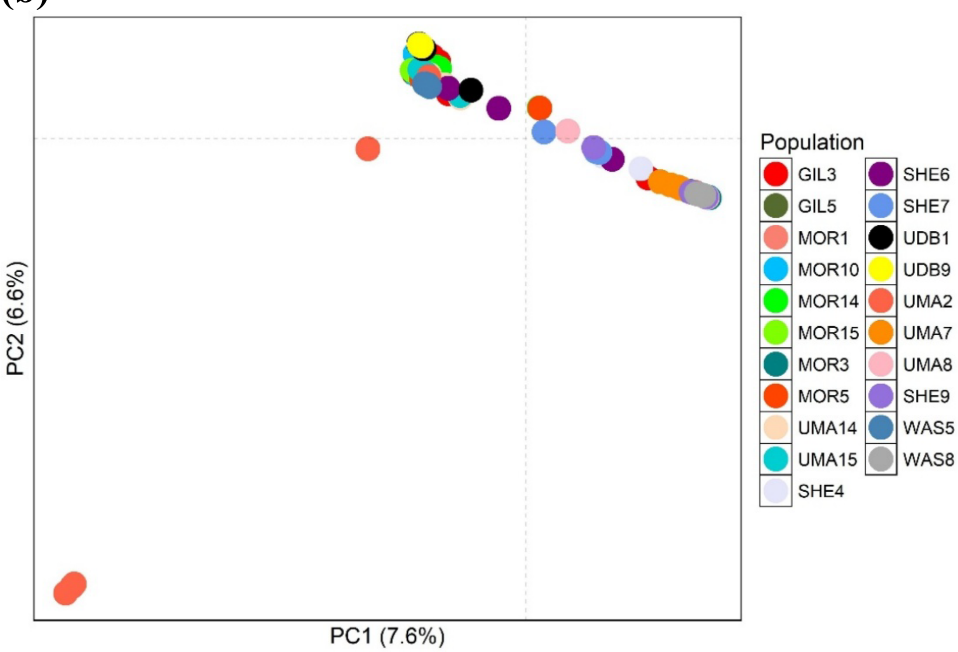

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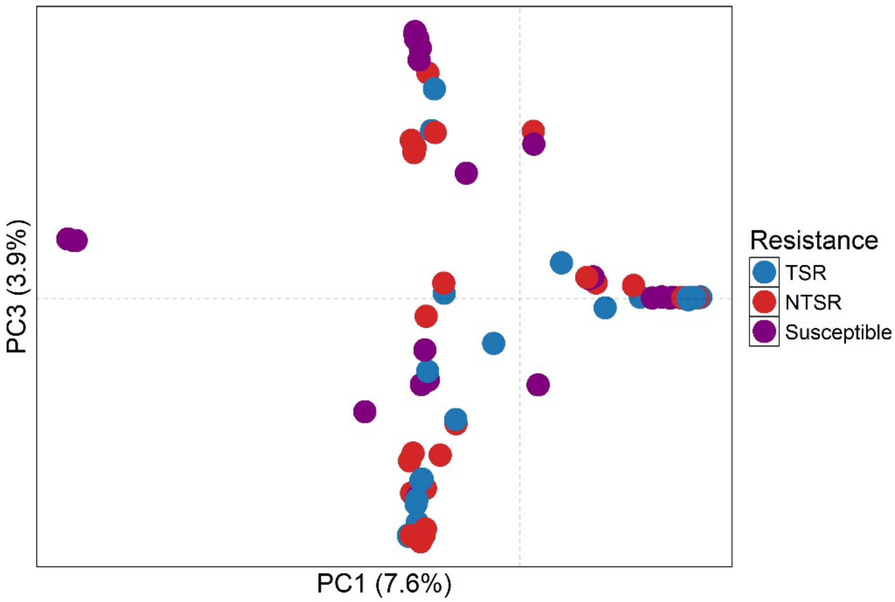
(c)



(d)

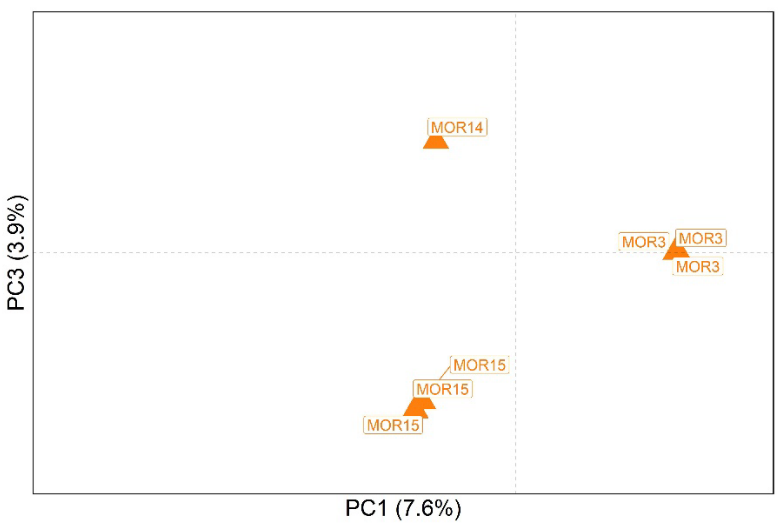


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Figure S1. Principal component analysis (PCA) of *Bromus tectorum* populations. (a) including *B. tectorum* populations and outgroup. (b) underscoring the separation of *B. tectorum* populations based on resistant individuals with target site mutations (TSR), resistant individuals without target site mutations (NTSR), and susceptible individuals. (c) PCA with individuals plotted between PC1 and PC2. (d) underscoring the separation of *B. tectorum* populations carrying the Pro-197-His mutation.

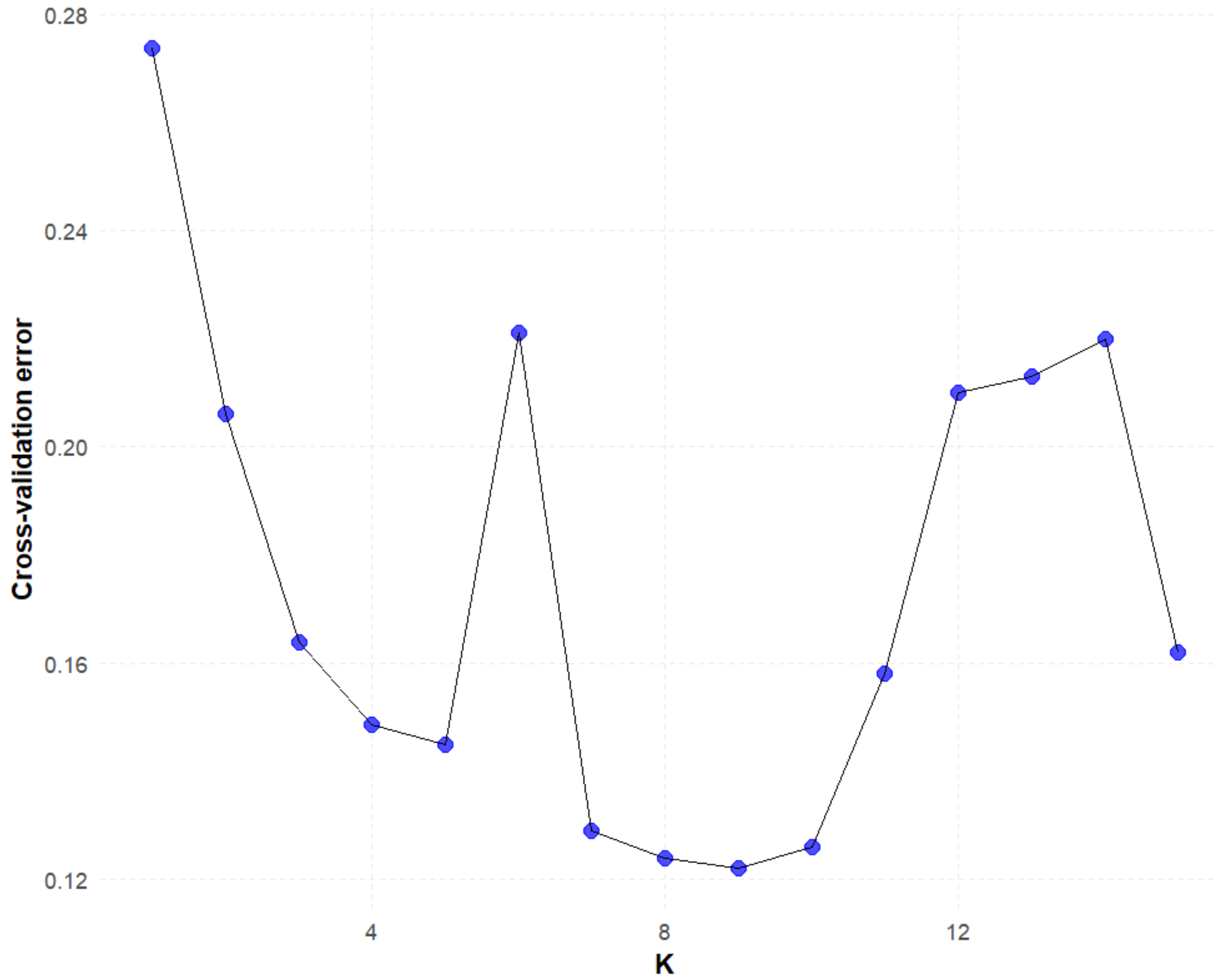

Figure S2. Cross-validation plot showing error versus *K*. The K with the best model fit minimizes error values.

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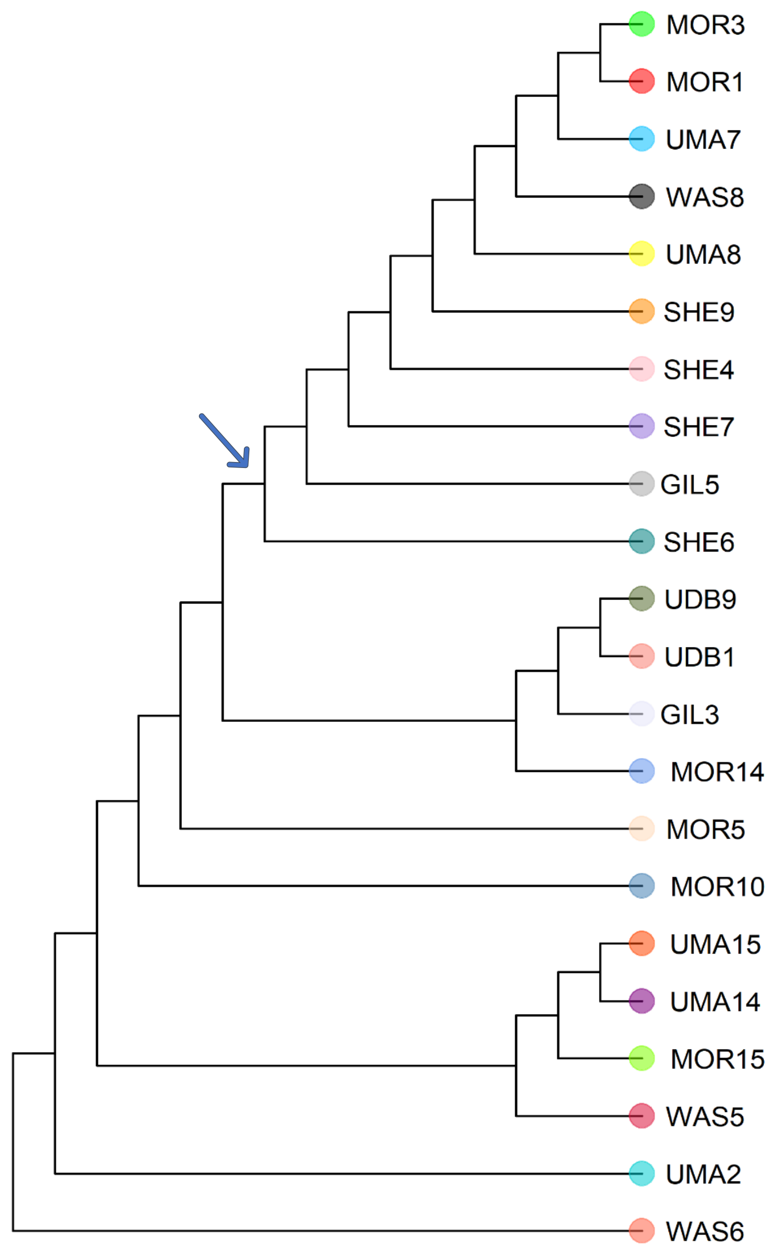

Figure S3. Rooted Neighbor-joining tree including the *B. tectorum* populations and the outgroup (*B. diandrus*; WAS6). The blue arrow highlights the base of the clade that includes the "blue" ADMIXTURE population.

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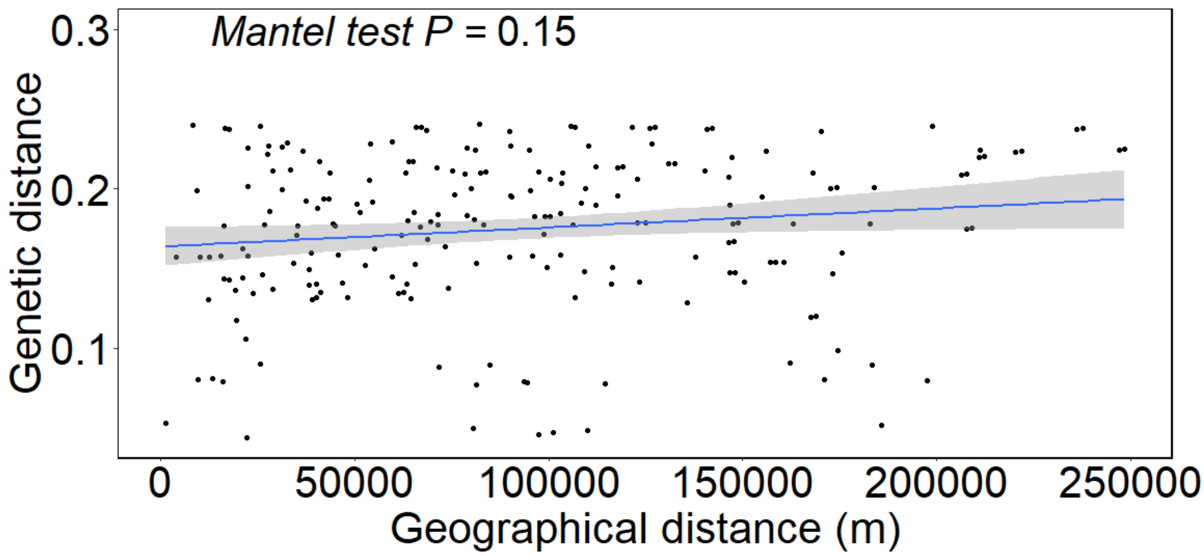

Figure S4. Isolation-by-distance plot with pairwise genetic and geographic distance between *B. tectorum* individuals. The Mantel test was performed with 10,000 simulations ($P = 0.15$).

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Table S1. Primers used for *ALS* gene sequencing.

Primers	Sequence (5'-3')	Ta (°C)[d]	Fragment (bp)	Position
Kumar_F[a]	GTCGACGTCTTCGCCTACC	68	1,271	Ala-122, Pro-197, and Ala-205
G_F1[b]	CGACGTCTTCGCCTACCC			
F_770[b]	CACCATCTACTGAATCGCTTGAG	66	762	Asp-376 and Arg-377
R_1533[b]	CATCAATGTCAACAACTGTAACACC			
Park_F[c]	GGGCAGCACCAGATGTGGGCG	62	556	Trp-574, Ser-653, and Gly-654
Kumar_R[a]	AATATTCGATCCTGCCATCA			

[a]Primer sequences previously described by Kumar & Jha (2017).
[b]Primer sequences designed using Primer 3 Plus software.
[c]Primer sequence previously described by Park & Mallory-Smith (2004).
[d]Primer annealing temperature.

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Table S2. *Bromus tectorum* populations collected from wheat and fine fescue fields included in the population genomics analysis.

Population	Latitude	Longitude	Sample number
GIL3	45.3879306	-120.1543083	4
GIL5	45.5751889	-120.1952083	4
MOR1	45.35834444	-119.8144778	5
MOR3	45.49412778	-119.6069194	5
MOR5	45.28898	-119.8833783	4
MOR10	45.508159	-120.006801	5
MOR14	45.436833	-119.882821	4
MOR15	45.5599667	-119.6548639	4
SHE4	45.5478167	-120.6940694	4
SHE6	45.4083139	-120.66705	4
SHE7	45.441375	-120.6466444	4
SHE9	45.569144	-120.813525	4
UDB1[1]	45.3138917	-118.015275	5
UDB9[1]	45.3096611	-117.9977138	5
UMA2	45.7457611	-119.3927333	5
UMA7	45.722886	-118.625071	4
UMA8	45.7877222	-118.4781667	4
UMA14	45.95583333	-118.8977778	4
UMA15	45.78555556	-118.8327778	5
WAS5	45.513	-121.163	4
WAS8	45.617	-121.009	5

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Table S3. Phenotype and sequence analyses of *B. tectorum* populations collected from wheat fields.[a]

Populations	ALS resistance status[b]	*ALS* mutations (frequency)	PSII resistance status[c]	*psbA* mutation (frequency)
GIL1	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
GIL2	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
GIL3	**Resistant**	**Pro-197-Leu (2/4)/Pro-197-Thr (1/4)**	**Resistant**	**Ser-264-Gly (4/4)**
GIL4	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
GIL5	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
GIL6	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
MOR1	**Resistant**	**Pro-197-Thr (2/4)**	**Susceptible**	**WT**
MOR2	**Resistant**	**Pro-197-Thr (1/4)**	**Susceptible**	**WT**
MOR3	**Resistant**	**Pro-197-Thr (1/4)/Pro-197-His (2/4)**	**Susceptible**	**WT**
MOR4	**Resistant**	**Pro-197-Leu (2/4)/Asp-376-Glu (1/4)**	**Susceptible**	**WT**
MOR5	**Susceptible**	**WT (4/4)**	**Susceptible**	**WT**
MOR6	**Resistant**	**Ser-653-Asn (4/4)**	**Susceptible**	**WT**
MOR7	**Resistant**	**Pro-197-His (1/4)**	**Susceptible**	**WT**
MOR8	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
MOR9	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
MOR10	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
MOR11	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
MOR13	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
MOR14	**Resistant**	**Pro-197-His (1/4)**	**Susceptible**	**WT**
MOR15	**Resistant**	**Pro-197-His (3/4)**	**Susceptible**	**WT**
SHE1	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
SHE2	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
SHE4	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
SHE5	**Resistant**	**Pro-197-Ser (2/4)**	**Susceptible**	**WT**
SHE6	**Resistant**	**Pro-197-Thr (3/4)**	**Resistant**	**Ser-264-Gly (4/4)**
SHE7	**Susceptible**	**WT (3/4)**	**Susceptible**	**WT**
SHE8	**Resistant**	**Pro-197-Leu (1/4)**	**Susceptible**	**WT**
SHE9	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
SHE10	**Susceptible**	**WT (4/4)**	**Susceptible**	**WT**
SHE11	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
SHE12	**Resistant**	**Ala-122-Thr (2/4)**	**Susceptible**	**WT**
UMA1	**Susceptible**	**WT (4/4)**	**Susceptible**	**WT**
UMA2	**Susceptible**	**WT (4/4)**	**Susceptible**	**WT**

UMA3	**Susceptible**	**WT (4/4)**	**Susceptible**	**WT**
UMA4	**Resistant**	**Pro-197-Leu (1/4)/Asp-376-Glu (1/4)/Trp-574-Leu (1/4)**	**Susceptible**	**WT**
UMA5	**Susceptible**	**WT (3/4)**	**Susceptible**	**WT**
UMA6	**Susceptible**	**WT (4/4)**	**Susceptible**	**WT**
UMA7	**Susceptible**	**WT (2/4)**	**Susceptible**	**WT**
UMA8	**Resistant**	**Ala-205-Val (2/4)/Pro-197-Leu (1/4)**	**Susceptible**	**WT**
UMA9	**Resistant**	**Asp-376-Glu (4/4)**	**Susceptible**	**WT**
UMA10	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
UMA11	**Resistant**	**Ala-205-Val (4/4)**	**Susceptible**	**WT**
UMA12	**Resistant**	**Ala-205-Val (2/4)**	**Susceptible**	**WT**
UMA13	**Susceptible**	**WT (4/4)**	**Susceptible**	**WT**
UMA14	**Resistant**	**NTSR (3/4)**	**Susceptible**	**WT**
UMA15	**Resistant**	**Pro-197-Thr (4/4)**	**Susceptible**	**WT**
WAS5	**Resistant**	**NTSR (4/4)**	**Susceptible**	**WT**
WAS7	**Resistant**	**Pro-197-Leu (2/4)**	**Susceptible**	**WT**
WAS8	**Resistant**	**Pro-197-Leu (3/4)**	**Susceptible**	**WT**

[a]Abbreviations: NTSR, non-target-site resistance (ALS-resistant individuals with no detectable mutations); WT, wild type.
[b]Sulfosulfuron was used in the ALS resistance testing.
[c]Metribuzin was used in the PSII resistance testing.