

SEQ ID NO: 3:

CCATG GCC GGC GCC GAG GAG ATC GTG CTG CAG CCC ATC AAG GAG ATC	47
Ala Gly Ala Glu Glu Ile Val Leu Gln Pro Ile Lys Glu Ile	
1 5 10	
TCC GGC ACC GTC AAG CTG CCG GGG TCC AAG TCG CTT TCC AAC CGG ATC	95
Ser Gly Thr Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile	
15 20 25 30	
CTC CTA CTC GCC GCC CTG TCC GAG GGG ACA ACA GTG GTT GAT AAC CTG	143
Leu Leu Leu Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu	
35 40 45	
CTG AAC AGT GAG GAT GTC CAC TAC ATG CTC GGG GCC TTG AGG ACT CTT	191
Leu Asn Ser Glu Asp Val His Tyr Met Leu Gly Ala Leu Arg Thr Leu	
50 55 60	
GGT CTC TCT GTC GAA GCG GAC AAA GCT GCC AAA AGA GCT GTA GTT GTT	239
Gly Leu Ser Val Glu Ala Asp Lys Ala Ala Lys Arg Val Val Val	
65 70 75	
GGC TGT GGT GGA AAG TTC CCA GTT GAG GAT GCT AAA GAG GAA GTG CAG	287
Gly Cys Gly Gly Lys Phe Pro Val Glu Asp Ala Lys Glu Glu Val Gln	
80 85 90	
CTC TTC TTG GGG AAT GCT GGA ACT GCA ATG CGG CCA TTG ACA GCA GCT	335
Leu Phe Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala	
95 100 105 110	
GTT ACT GCT GCT GGT GGA AAT GCA ACT TAC GTG CTT GAT GGA GTA CCA	383
Val Thr Ala Ala Gly Gly Asn Ala Thr Tyr Val Leu Asp Gly Val Pro	
115 120 125	
AGA ATG AGG GAG AGA CCC ATT GGC GAC TTG GTT GTC GGA TTG AAG CAG	431
Arg Met Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Lys Gln	
130 135 140	
CTT GGT GCA GAT GTT GAT TGT TTC CTT GGC ACT GAC TGC CCA CCT GTT	479
Leu Gly Ala Asp Val Asp Cys Phe Leu Gly Thr Asp Cys Pro Pro Val	
145 150 155	
CGT GTC AAT GGA ATC GGA GGG CTA CCT GGT GGC AAG GTC AAG CTG TCT	527
Arg Val Asn Gly Ile Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser	
160 165 170	
GGC TCC ATC AGC AGT CAG TAC TTG AGT GCC TTG CTG ATG GCT GCT CCT	575
Gly Ser Ile Ser Ser Gln Tyr Leu Ser Ala Leu Leu Met Ala Ala Pro	
175 180 185 190	
TTG GCT CTT GGG GAT GTG GAG ATT GAA ATC ATT GAT AAA TTA ATC TCC	623
Leu Ala Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser	
195 200 205	
ATT CCG TAC GTC GAA ATG ACA TTG AGA TTG ATG GAG CGT TTT GGT GTG	671
Ile Pro Tyr Val Glu Met Thr Leu Arg Leu Met Glu Arg Phe Gly Val	
210 215 220	
AAA GCA GAG CAT TCT GAT AGC TGG GAC AGA TTC TAC ATT AAG GGA GGT	719
Lys Ala Glu His Ser Asp Ser Trp Asp Arg Phe Tyr Ile Lys Gly Gly	
225 230 235	
CAA AAA TAC AAG TCC CCT AAA AAT GCC TAT GTT GAA GGT GAT GCC TCA	767
Gln Lys Tyr Lys Ser Pro Lys Asn Ala Tyr Val Glu Gly Asp Ala Ser	
240 245 250	

FEUILLE DE REMPLACEMENT (REGLE 26)

SEQ ID NO: 3(suite)

AGC Ser 255	GCA Ala	AGC Ser	TAT Tyr	TTC Phe	TTG Leu 260	GCT Ala	GGT Gly	GCT Ala	GCA Ala	ATT Ile 265	ACT Thr	GGA Gly	GGG Gly	ACT Thr	GTG Val 270	815
ACT Thr	GTG Val	GAA Glu	GGT Gly	TGT Cys 275	GGC Gly	ACC Thr	ACC Thr	AGT Ser	TTG Leu 280	CAG Gln	GGT Gly	GAT Asp	GTG Val	AAG Lys 285	TTT Phe	863
GCT Ala	GAG Glu	GTA Val	CTG Leu 290	GAG Glu	ATG Met	ATG Met	GGA Gly	GCG Ala 295	AAG Lys	GTT Val	ACA Thr	TGG Trp	ACC Thr 300	GAG Glu	ACT Thr	911
AGC Ser	GTA Val	ACT Thr 305	GTT Val	ACT Thr	GGC Gly	CCA Pro	CCG Pro 310	CGG Arg	GAG Glu	CCA Pro	TTT Phe 315	GGG Gly 315	AGG Arg	AAA Lys	CAC His	959
CTC Leu 320	AAG Lys	GCG Ala	ATT Ile	GAT Asp	GTC Val	AAC Asn 325	ATG Met	AAC Asn	AAG Lys	ATG Met 330	CCT Pro 330	GAT Asp	GTC Val	GCC Ala	ATG Met	1007
ACT Thr 335	CTT Leu	GCT Ala	GTG Val	GTT Val	GCC Ala 340	CTC Leu	TTT Phe	GCC Ala	GAT Asp	GGC Gly 345	CCG Pro	ACA Thr	GCC Ala	ATC Ile	AGA Arg 350	1055
GAC Asp	GTG Val	GCT Ala	TCC Ser	TGG Trp 355	AGA Arg	GTA Val	AAG Lys	GAG Glu	ACC Thr 360	GAG Glu	AGG Arg	ATG Met	GTT Val	GCG Ala 365	ATC Ile	1103
CGG Arg	ACG Thr	GAG Glu	CTA Leu 370	ACC Thr	AAG Lys	CTG Leu	GGA Gly	GCA Ala 375	TCT Ser	GTT Val	GAG Glu	GAA Glu	GGG Gly 380	CCG Pro	GAC Asp	1151
TAC Tyr	TGC Cys	ATC Ile 385	ATC Ile	ACG Thr	CCG Pro	CCG Pro	GAG Glu 390	AAG Lys	CTG Leu	AAC Asn	GTG Val	ACG Thr 395	GCG Ala	ATC Ile	GAC Asp	1199
ACG Thr 400	TAC Tyr	GAC Asp	GAC Asp	CAC His	AGG Arg	ATG Met 405	GCC Ala	ATG Met	GCC Ala	TTC Phe 410	TCC Ser 410	CTT Leu	GCC Ala	GCC Ala	TGT Cys	1247
GCC Ala 415	GAG Glu	GTC Val	CCC Pro	GTC Val	ACC Thr 420	ATC Ile	CGG Arg	GAC Asp	CCT Pro	GGG Gly 425	TGC Cys	ACC Thr	CGG Arg	AAG Lys	ACC Thr 430	1295
TTC Phe	CCC Pro	GAC Asp	TAC Tyr	TTC Phe 435	GAT Asp	GTG Val	CTG Leu	AGC Ser	ACT Thr 440	TTC Phe	GTC Val	AAG Lys	AAT Asn			1337
TAA																1340

SEQ ID NO: 4:

Ala Gly Ala Glu Glu Ile Val Leu Gln Pro Ile Lys Glu Ile Ser Gly
 1 5 10 15
 Thr Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu
 20 25 30
 Leu Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu Asn
 35 40 45
 Ser Glu Asp Val His Tyr Met Leu Gly Ala Leu Arg Thr Leu Gly Leu
 50 55 60
 Ser Val Glu Ala Asp Lys Ala Ala Lys Arg Ala Val Val Val Gly Cys
 65 70 75 80
 Gly Gly Lys Phe Pro Val Glu Asp Ala Lys Glu Glu Val Gln Leu Phe
 85 90 95
 Leu Gly Asn Ala Gly Thr Ala Met Arg Pro Leu Thr Ala Ala Val Thr
 100 105 110
 Ala Ala Gly Gly Asn Ala Thr Tyr Val Leu Asp Gly Val Pro Arg Met
 115 120 125
 Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Lys Gln Leu Gly
 130 135 140
 Ala Asp Val Asp Cys Phe Leu Gly Thr Asp Cys Pro Pro Val Arg Val
 145 150 155 160
 Asn Gly Ile Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser
 165 170 175
 Ile Ser Ser Gln Tyr Leu Ser Ala Leu Leu Met Ala Ala Pro Leu Ala
 180 185 190
 Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Ile Pro
 195 200 205
 Tyr Val Glu Met Thr Leu Arg Leu Met Glu Arg Phe Gly Val Lys Ala
 210 215 220
 Glu His Ser Asp Ser Trp Asp Arg Phe Tyr Ile Lys Gly Gly Gln Lys
 225 230 235 240
 Tyr Lys Ser Pro Lys Asn Ala Tyr Val Glu Gly Asp Ala Ser Ser Ala
 245 250 255
 Ser Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Gly Thr Val Thr Val
 260 265 270
 Glu Gly Cys Gly Thr Thr Ser Leu Gln Gly Asp Val Lys Phe Ala Glu
 275 280 285
 Val Leu Glu Met Met Gly Ala Lys Val Thr Trp Thr Glu Thr Ser Val
 290 295 300
 Thr Val Thr Gly Pro Pro Arg Glu Pro Phe Gly Arg Lys His Leu Lys
 305 310 315 320
 Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
 325 330 335
 Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Ala Ile Arg Asp Val
 340 345 350

FEUILLE DE REMPLACEMENT (REGLE 26)

SEQ ID NO: 4 (suite)

Ala	Ser	Trp	Arg	Val	Lys	Glu	Thr	Glu	Arg	Met	Val	Ala	Ile	Arg	Thr
		355					360					365			
Glu	Leu	Thr	Lys	Leu	Gly	Ala	Ser	Val	Glu	Glu	Gly	Pro	Asp	Tyr	Cys
	370					375					380				
Ile	Ile	Thr	Pro	Pro	Glu	Lys	Leu	Asn	Val	Thr	Ala	Ile	Asp	Thr	Tyr
385					390					395					400
Asp	Asp	His	Arg	Met	Ala	Met	Ala	Phe	Ser	Leu	Ala	Ala	Cys	Ala	Glu
				405					410					415	
Val	Pro	Val	Thr	Ile	Arg	Asp	Pro	Gly	Cys	Thr	Arg	Lys	Thr	Phe	Pro
			420					425					430		
Asp	Tyr	Phe	Asp	Val	Leu	Ser	Thr	Phe	Val	Lys	Asn				
		435					440								

SEQ ID NO: 5:

CCATG GCC GGC GCC GAG GAG ATC GTG CTG CAG CCC ATC AAG GAG ATC	47
Ala Gly Ala Glu Glu Ile Val Leu Gln Pro Ile Lys Glu Ile	
1 5 10	
TCC GGC ACC GTC AAG CTG CCG GGG TCC AAG TCG CTT TCC AAC CGG ATC	95
Ser Gly Thr Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile	
15 20 25 30	
CTC CTA CTC GCC GCC CTG TCC GAG GGG ACA ACA GTG GTT GAT AAC CTG	143
Leu Leu Leu Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu	
35 40 45	
CTG AAC AGT GAG GAT GTC CAC TAC ATG CTC GGG GCC TTG AGG ACT CTT	191
Leu Asn Ser Glu Asp Val His Tyr Met Leu Gly Ala Leu Arg Thr Leu	
50 55 60	
GGT CTC TCT GTC GAA GCG GAC AAA GCT GCC AAA AGA GCT GTA GTT GTT	239
Gly Leu Ser Val Glu Ala Asp Lys Ala Ala Lys Arg Ala Val Val Val	
65 70 75	
GGC TGT GGT GGA AAG TTC CCA GTT GAG GAT GCT AAA GAG GAA GTG CAG	287
Gly Cys Gly Gly Lys Phe Pro Val Glu Asp Ala Lys Glu Glu Val Gln	
80 85 90	
CTC TTC TTG GGG AAT GCT GGA ATC GCA ATG CGG TCC TTG ACA GCA GCT	335
Leu Phe Leu Gly Asn Ala Gly Ile Ala Met Arg Ser Leu Thr Ala Ala	
95 100 105 110	
GTT ACT GCT GCT GGT GGA AAT GCA ACT TAC GTG CTT GAT GGA GTA CCA	383
Val Thr Ala Ala Gly Gly Asn Ala Thr Tyr Val Leu Asp Gly Val Pro	
115 120 125	
AGA ATG AGG GAG AGA CCC ATT GGC GAC TTG GTT GTC GGA TTG AAG CAG	431
Arg Met Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Lys Gln	
130 135 140	
CTT GGT GCA GAT GTT GAT TGT TTC CTT GGC ACT GAC TGC CCA CCT GTT	479
Leu Gly Ala Asp Val Asp Cys Phe Leu Gly Thr Asp Cys Pro Pro Val	
145 150 155	
CGT GTC AAT GGA ATC GGA GGG CTA CCT GGT GGC AAG GTC AAG CTG TCT	527
Arg Val Asn Gly Ile Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser	
160 165 170	
GGC TCC ATC AGC AGT CAG TAC TTG AGT GCC TTG CTG ATG GCT GCT CCT	575
Gly Ser Ile Ser Ser Gln Tyr Leu Ser Ala Leu Leu Met Ala Ala Pro	
175 180 185 190	
TTG GCT CTT GGG GAT GTG GAG ATT GAA ATC ATT GAT AAA TTA ATC TCC	623
Leu Ala Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser	
195 200 205	
ATT CCG TAC GTC GAA ATG ACA TTG AGA TTG ATG GAG CGT TTT GGT GTG	671
Ile Pro Tyr Val Glu Met Thr Leu Arg Leu Met Glu Arg Phe Gly Val	
210 215 220	
AAA GCA GAG CAT TCT GAT AGC TGG GAC AGA TTC TAC ATT AAG GGA GGT	719
Lys Ala Glu His Ser Asp Ser Trp Asp Arg Phe Tyr Ile Lys Gly Gly	
225 230 235	
CAA AAA TAC AAG TCC CCT AAA AAT GCC TAT GTT GAA GGT GAT GCC TCA	767
Gln Lys Tyr Lys Ser Pro Lys Asn Ala Tyr Val Glu Gly Asp Ala Ser	
240 245 250	

SEQ ID NO: 5 (suite)

AGC GCA AGC TAT TTC TTG GCT GGT GCT GCA ATT ACT GGA GGG ACT GTG	815
Ser Ala Ser Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Gly Thr Val	
255 260 265 270	
ACT GTG GAA GGT TGT GGC ACC ACC AGT TTG CAG GGT GAT GTG AAG TTT	863
Thr Val Glu Gly Cys Gly Thr Thr Ser Leu Gln Gly Asp Val Lys Phe	
270 275 280 285	
GCT GAG GTA CTG GAG ATG ATG GGA GCG AAG GTT ACA TGG ACC GAG ACT	911
Ala Glu Val Leu Glu Met Met Gly Ala Lys Val Thr Trp Thr Glu Thr	
290 295 300	
AGC GTA ACT GTT ACT GGC CCA CCG CGG GAG CCA TTT GGG AGG AAA CAC	959
Ser Val Thr Val Thr Gly Pro Pro Arg Glu Pro Phe Gly Arg Lys His	
305 310 315	
CTC AAG GCG ATT GAT GTC AAC ATG AAC AAG ATG CCT GAT GTC GCC ATG	1007
Leu Lys Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met	
320 325 330	
ACT CTT GCT GTG GTT GCC CTC TTT GCC GAT GGC CCG ACA GCC ATC AGA	1055
Thr Leu Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Ala Ile Arg	
335 340 345 350	
GAC GTG GCT TCC TGG AGA GTA AAG GAG ACC GAG AGG ATG GTT GCG ATC	1103
Asp Val Ala Ser Trp Arg Val Lys Glu Thr Glu Arg Met Val Ala Ile	
355 360 365	
CGG ACG GAG CTA ACC AAG CTG GGA GCA TCT GTT GAG GAA GGG CCG GAC	1151
Arg Thr Glu Leu Thr Lys Leu Gly Ala Ser Val Glu Glu Gly Pro Asp	
370 375 380	
TAC TGC ATC ATC ACG CCG CCG GAG AAG CTG AAC GTG ACG GCG ATC GAC	1199
Tyr Cys Ile Ile Thr Pro Pro Glu Lys Leu Asn Val Thr Ala Ile Asp	
385 390 395	
ACG TAC GAC GAC CAC AGG ATG GCG ATG GCC TTC TCC CTT GCC GCC TGT	1247
Thr Tyr Asp Asp His Arg Met Ala Met Ala Phe Ser Leu Ala Ala Cys	
400 405 410	
GCC GAG GTC CCC GTC ACC ATC CGG GAC CCT GGG TGC ACC CGG AAG ACC	1295
Ala Glu Val Pro Val Thr Ile Arg Asp Pro Gly Cys Thr Arg Lys Thr	
415 420 425 430	
TTC CCC GAC TAC TTC GAT GTG CTG AGC ACT TTC GTC AAG AAT	1337
Phe Pro Asp Tyr Phe Asp Val Leu Ser Thr Phe Val Lys Asn	
435 440	
TAA	1340

SEQ ID NO: 6:

Ala Gly Ala Glu Glu Ile Val Leu Gln Pro Ile Lys Glu Ile Ser Gly
 1 5 10 15
 Thr Val Lys Leu Pro Gly Ser Lys Ser Leu Ser Asn Arg Ile Leu Leu
 20 25 30
 Leu Ala Ala Leu Ser Glu Gly Thr Thr Val Val Asp Asn Leu Leu Asn
 35 40 45
 Ser Glu Asp Val His Tyr Met Leu Gly Ala Leu Arg Thr Leu Gly Leu
 50 55 60
 Ser Val Glu Ala Asp Lys Ala Ala Lys Arg Ala Val Val Val Gly Cys
 65 70 75 80
 Gly Gly Lys Phe Pro Val Glu Asp Ala Lys Glu Glu Val Gln Leu Phe
 85 90 95
 Leu Gly Asn Ala Gly Ile Ala Met Arg Ser Leu Thr Ala Ala Val Thr
 100 105 110
 Ala Ala Gly Gly Asn Ala Thr Tyr Val Leu Asp Gly Val Pro Arg Met
 115 120 125
 Arg Glu Arg Pro Ile Gly Asp Leu Val Val Gly Leu Lys Gln Leu Gly
 130 135 140
 Ala Asp Val Asp Cys Phe Leu Gly Thr Asp Cys Pro Pro Val Arg Val
 145 150 155 160
 Asn Gly Ile Gly Gly Leu Pro Gly Gly Lys Val Lys Leu Ser Gly Ser
 165 170 175
 Ile Ser Ser Gln Tyr Leu Ser Ala Leu Leu Met Ala Ala Pro Leu Ala
 180 185 190
 Leu Gly Asp Val Glu Ile Glu Ile Ile Asp Lys Leu Ile Ser Ile Pro
 195 200 205
 Tyr Val Glu Met Thr Leu Arg Leu Met Glu Arg Phe Gly Val Lys Ala
 210 215 220
 Glu His Ser Asp Ser Trp Asp Arg Phe Tyr Ile Lys Gly Gly Gln Lys
 225 230 235 240
 Tyr Lys Ser Pro Lys Asn Ala Tyr Val Glu Gly Asp Ala Ser Ser Ala
 245 250 255
 Ser Tyr Phe Leu Ala Gly Ala Ala Ile Thr Gly Gly Thr Val Thr Val
 260 265 270
 Glu Gly Cys Gly Thr Thr Ser Leu Gln Gly Asp Val Lys Phe Ala Glu
 275 280 285
 Val Leu Glu Met Met Gly Ala Lys Val Thr Trp Thr Glu Thr Ser Val
 290 295 300
 Thr Val Thr Gly Pro Pro Arg Glu Pro Phe Gly Arg Lys His Leu Lys
 305 310 315 320
 Ala Ile Asp Val Asn Met Asn Lys Met Pro Asp Val Ala Met Thr Leu
 325 330 335
 Ala Val Val Ala Leu Phe Ala Asp Gly Pro Thr Ala Ile Arg Asp Val
 340 345 350

FEUILLE DE REMPLACEMENT (REGLE 26)

SEQ ID NO: 6 (suite)

Ala	Ser	Trp	Arg	Val	Lys	Glu	Thr	Glu	Arg	Met	Val	Ala	Ile	Arg	Thr
		355					360					365			
Glu	Leu	Thr	Lys	Leu	Gly	Ala	Ser	Val	Glu	Glu	Gly	Pro	Asp	Tyr	Cys
	370					375					380				
Ile	Ile	Thr	Pro	Pro	Glu	Lys	Leu	Asn	Val	Thr	Ala	Ile	Asp	Thr	Tyr
385					390					395					400
Asp	Asp	His	Arg	Met	Ala	Met	Ala	Phe	Ser	Leu	Ala	Ala	Cys	Ala	Glu
				405					410					415	
Val	Pro	Val	Thr	Ile	Arg	Asp	Pro	Gly	Cys	Thr	Arg	Lys	Thr	Phe	Pro
			420					425					430		
Asp	Tyr	Phe	Asp	Val	Leu	Ser	Thr	Phe	Val	Lys	Asn				
		435					440								

Revendications

5

1. Gène chimère comprenant au moins deux gènes chimères élémentaires comprenant chacun des éléments de régulation nécessaires à sa transcription dans les plantes et une
séquence codante codant pour une enzyme conférant aux plantes la tolérance à un
10 herbicide, caractérisé en ce que l'une des séquences codantes code pour l'hydroxy-phényl
pyruvate dioxygénase (HPPD).
2. Gène chimère selon la revendication 1, caractérisé en ce qu'il comprend en outre un
troisième gène chimère contenant une séquence codante pour une enzyme conférant aux
15 plantes une tolérance herbicide.
3. Gène chimère selon l'une des revendications 1 et 2, caractérisé en ce que la seconde
séquence codante est issu d'un gène de nitrilase *Klebsiella sp.* conférant une tolérance à un
herbicide de la famille des dihalogénohydroxybenzonitriles.
20
4. Gène chimère selon la revendication 3, caractérisé en ce que l'herbicide est le bromoxynil.
5. Gène chimère selon la revendication 3, caractérisé en ce que l'herbicide est l'ioxynil.
- 25 6. Gène chimère selon l'une des revendications 2 à 5, caractérisé en ce que la seconde
séquence codante code pour une tolérance au glyphosate.
7. Gène chimère selon l'une des revendications 2 à 6, caractérisé en ce que la seconde
séquence codante code pour une EPSPS conférant une tolérance à un herbicide inhibiteur
30 de l'EPSPS.
8. Gène chimère selon la revendication 7, caractérisé en ce que la seconde séquence codante
code pour une EPSPS conférant une tolérance au glyphosate.
- 35 9. Gène chimère selon la revendication 6, caractérisé en ce que la seconde séquence codante
code pour la glyphosate oxydoréductase, enzyme de détoxification du glyphosate.

10. Gène chimère selon l'une des revendications 1 à 9, caractérisé en ce que la séquence codante pour l'HPPD est issue de *Pseudomonas sp.*
- 5 11. Gène chimère selon la revendication 10, caractérisé en ce que la séquence codante pour l'HPPD est issue de *Pseudomonas fluorescens.*
12. Gène chimère selon l'une des revendications 1 à 9, caractérisé en ce que la séquence codante pour l'HPPD est d'origine végétale.
- 10 13. Gène chimère selon la revendication 12, caractérisé en ce que la séquence codante pour l'HPPD est issue de *Arabidopsis thaliana.*
14. Gène chimère selon la revendication 11, caractérisé en ce que la séquence codante pour l'HPPD est issue de *Daucus carota.*
- 15 15. Vecteur caractérisé en ce qu'il comprend un gène chimère selon l'une des revendications 1 à 14.
16. Vecteur selon la revendication 15, caractérisé en ce qu'il est constitué par un plasmide.
- 20 17. Cellule végétale, caractérisée en ce qu'elle contient au moins deux gènes contenant chacun une séquence codante codant pour une enzyme conférant aux plantes la tolérance à un herbicide, dont l'un est un inhibiteur de l'hydroxy-phényl pyruvate dioxygénase (HPPD).
- 25 18. Cellule végétale selon la revendication 17, caractérisée en ce qu'elle contient trois gènes chimères élémentaires contenant chacun des éléments de régulation et une séquence codante codant pour une enzyme conférant aux plantes la tolérance à un herbicide.
- 30 19. Cellule végétale selon l'une des revendications 17 et 18, caractérisée en ce qu'elle contient au moins un gène chimère, selon l'une des revendications 1 à 14.
- 35 20. Plante, caractérisée en ce qu'elle contient une cellule végétale selon l'une des revendications 17 à 19.

21. Procédé de transformation des plantes pour les rendre tolérantes à au moins d eux herbicides, caractérisé en ce qu'on insère dans une cellule végétale un gène selon les revendications 1 à 14 et que les cellules transformées sont soumises à une régénération.
- 5 22. Procédé d'obtention de plantes à tolérance herbicide multiple par transgénèse des plantes, caractérisé en ce que:
- dans une première étape, on insère dans plusieurs cellules respectivement un des gènes élémentaires contenant chacun des éléments de régulation nécessaires à sa
 - 10 transcription dans les plantes et une séquence codante codant pour une enzyme conférant aux plantes la tolérance à un herbicide, et que
 - ensuite les plantes sont croisées pour obtenir des plantes à tolérance multiple.
23. Procédé de traitement herbicide de plantes selon la revendication 20, caractérisé en ce qu'on applique au moins deux herbicides.
- 15 24. Procédé selon la revendication 23, caractérisé en ce qu'on applique trois herbicides.
25. Procédé selon l'une des revendications 21 à 24, caractérisé en ce que l'un des herbicides est un inhibiteur de l'HPPD.
- 20 26. Procédé selon l'une des revendications 21 à 25, caractérisé en ce que les deux herbicides sont appliqués simultanément.
27. Procédé selon la revendication 26, caractérisé en ce que les deux herbicides sont
- 25 appliqués sous la forme d'une seule composition prête à l'emploi.
28. Procédé selon la revendication 25, caractérisé en ce que les deux herbicides sont appliqués sous la forme d'un mélange extemporané.
- 30 29. Procédé l'une des revendications 22 à 24, caractérisé en ce que les deux herbicides sont appliqués successivement.
30. Procédé selon l'une des revendications 22 à 29, caractérisé en ce que l'herbicide inhibiteur de l'HPPD est l'isoxaflutole.
- 35 31. Procédé selon l'une des revendications 22 à 29, caractérisé en ce que l'herbicide inhibiteur de l'HPPD est la sulcotrione.

32. Procédé selon l'une des revendications 22 à 31, caractérisé en ce que l'herbicide appartient à la famille des dihydrogènohydroxybenzonitriles.
33. Procédé selon la revendication 32, caractérisé en ce que l'herbicide est choisi dans le
5 groupe comprenant le bromoxynil et l'ioxynil
34. Procédé selon l'une des revendications 22 à 33, caractérisé en ce que l'herbicide inhibiteur de l'EPSPS est le glyphosate ou le sulfosate.

G G N G A Y Y T N T A Y G A R A A Y C C N A T G G	G N Y T N A T G G G N T T Y G A R T T Y A T H G A	R Y T N G C N W S N C C N A C N C N A A Y A C N	75
A D L Y E N P M G L M G F E F I E L A S P T P N T			
Y T N G A R C C N A T T T Y G A R A T H A T G G	G N T T Y A C N A A R G T N G C N A C N C A Y M G	M W S N A A R G A Y G T N C A Y Y T N T A Y M G N	150
L E P I F E I M G F T K V A T H R S K D V H L Y R			
C A R G G N G C N A T H A A Y Y T N A T H Y T N A	A Y A A Y G A R C C N C A Y W S N G T N G C N W S	N T A Y T T Y G C N G C N G A R C A Y G G N C C N	225
Q G A I N L I L N N E P H S V A S Y F A A E H G P			
W S N G T N T G Y G G N A T G G C N T T Y M G N G	T N A A R G A Y W S N C A R A A R G C N T A Y A A	R M G N G C N Y T N G A R Y T N G G N G C N C A R	300
S V C G M A F R V K D S Q K A Y K R A L E L G A Q			
C C N A T H C A Y A T H G A R A C N G G N C C N A	T G G A R Y T N A A Y Y T N C C N G C N A T H A A	R G G N A T H G G N G G N G C N C C N Y T N T A Y	375
P I H I E T G P M E L N L P A I K G I G G A P L Y			
Y T H A T H G A Y M G N T T Y G G N G A R G G N W	S N W S N A T H T A Y G A Y A T H G A Y T T Y G T	N T T Y Y T N G A R G G N G T N G A Y M G N C A Y	450
K I D R F G E G S S I Y D I D F V F L E G V D R H			
C C N G T N G G N G C N G G N Y T H A A R A T H A	T H G A Y C A Y Y T N A C N C A Y A A Y G T N T A	Y M G N G G N M G N A T G G C N T A Y T G G G C N	525
P V G A G L K I I D H L T H N V Y R G R M A Y W A			
A A Y T T Y T A Y G A R A A R Y T N T T Y A A Y T	T Y M G N G A R A T H M G N T A Y T T Y G A Y A T	H A A R G G N G A R T A Y A C N G G N Y T N A C N	600
N F Y E K L F N F R E I R Y F D I K G E Y T G L T			
W S N A A R G C N A T G A C N G C N C N G A Y G	G N A T G A T H M G N A T H C C N Y T N A A Y G A	R G A R W S N W S N A A R G G N G C N G N C A R	675
S K A M T A P D G M I R I P L N E E S S K G A G Q			

1/5

Fig 1

ATHGARGARTTYYTNATGCARTTYA	AYGGNGARGGNATHCARCAYGTNGC	NTTYYTNWSNGAYGAYTNAATHAAR	750
I E E F L M Q F N	G E G I Q H V A F L S D D L I K		
ACNTGGGAYCAYTNAARWSNATHG	GNATGNGNTTYATGACNGCNCNC	NGAYACNTAYTAYGARATGYTNGAR	825
T W D H L K S I G	M R F M T A P P D T Y Y E M L E		
GGNMGNYTHCCNAAYCAYGGNGARC	CNGTNGGNGARYTNCARGCNMGNGG	NATHYTNYTNGAYGGNWSNWSNGAR	900
G R L P N H G E P	V G E L Q A R G I L L D G S S E		
WSNGGNGAYAARMGNYTNYTNYTNC	ARATHTTYWSNGARACNYTNATGGG	NCCNGTNTTYTYGARTTYATHCAR	975
S G D K R L L L Q	I F S E T L M G P V F F E F I Q		
MGNAARGGNGAYGAYGGNTTYGGNG	ARGGNAAYTTYAARGCNYTNTTYGA	RWSNATHGARMGNGAYCARGTNMGN	1050
R K G D D G F G E	G N F K A L F E S I E R D Q V R		
MGNGGNGTNYTNWSNACNGAY			1071
R G V L S T D			

Fig 1(suite)

3/5

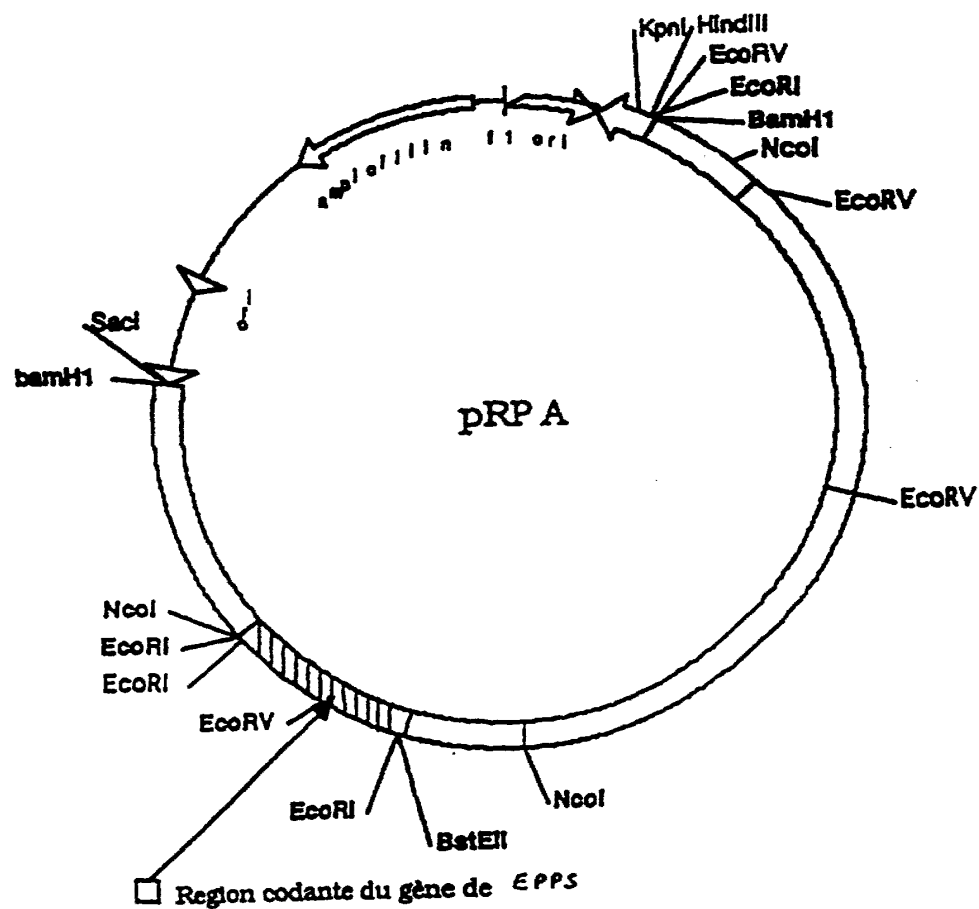


Fig 2

FEUILLE DE REMPLACEMENT (REGLE 26)

Consensus	.ADLYENPMG	LMGFEFIE.A	SPTP.TLEPI	FEIMGFTKVA	THRSK.VHLY	50
<i>P. fluorescens</i>	M.....	F.	G....		N....	50
<i>Pseudomonas sp.</i>	-.....	L.	N....		D....	49
Consensus	RQG.INLILN	NEP.S.ASYF	AAEHGPSVCG	MAFRVKDSQK	AY.RALELGA	100
<i>P. fluorescens</i>	...E.....	...N.I....			..N.....	100
<i>Pseudomonas sp.</i>	...A.....	...H.V....			..K.....	99
Consensus	QPIHI.TGPM	ELNLPAIKGI	GGAPLYLIDR	FGEGSSIIYDI	DFV.LEGV.R	150
<i>P. fluorescens</i>	D....				...Y....E.	150
<i>Pseudomonas sp.</i>	E....				...F....D.	149
Consensus	.PVGAGLK.I	DHLTHNVYRG	RM.YMANFYE	KLFNFRE.RY	FDIKGEYTG	200
<i>P. fluorescens</i>	N.....V.		..V.....	A..		200
<i>Pseudomonas sp.</i>	H.....I.		..A.....	I..		199
Consensus	TSKAM.APDG	MIRIPLNEES	SKGAGQIEEF	LMQFNQEGIQ	HVAFL.DDL.	250
<i>P. fluorescens</i>	S....				T...V	250
<i>Pseudomonas sp.</i>	T....				S...I	249

Fig 3

Consensus	KTWD.LK.IG	HRFMTAPPDT	YYENLEGRLP	.HGEPV..LQ	ARGILLDGSS	300
<i>P. fluorescens</i>	A..K..			D.....DQ..		300
<i>Pseudomonas sp.</i>	H..S..			N.....GE..		299
Consensus	..GDKRLLQ	IFSETLMGPV	FFEFIQRKGD	DGFGEGNFKA	LFESIERDQV	350
<i>P. fluorescens</i>	VE.....					350
<i>Pseudomonas sp.</i>	ES.....					349
Consensus	RRGYL..D					358
<i>P. fluorescens</i>	TÅ.					358
<i>Pseudomonas sp.</i>	ST.					357

Fig 3 (suite)

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(71) Applicant: THE UNITED STATES OF AMERICA, AS REPRESENTED BY THE SECRETARY OF AGRICULTURE [US/US]; 1400 Independence Avenue, S.W., Washington, DC 20250-0302 (US).

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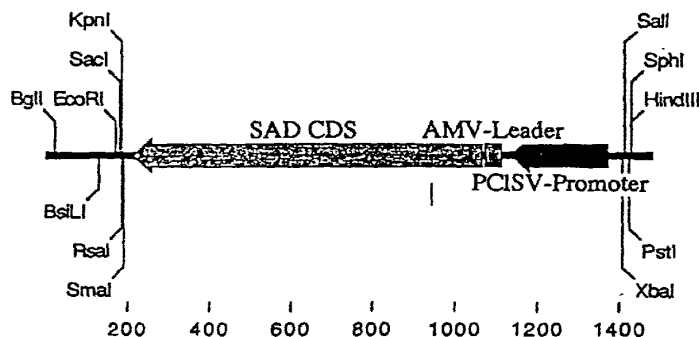
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(72) Inventors: OLIVER, Melvin, J.; 5728 72nd Street, Lubbock, TX 79424 (US). BURKE, John, J.; 5412 79th Street, Lubbock, TX 79424 (US). VELTEN, Jeffrey, P.; 5722 70th Place, Lubbock, TX 79424 (US).

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(74) Agents: PENDORF, Stephan, A. et al.; Pendorf & Cutliff, P.O. Box 20445, Tampa, FL 33622-0445 (US).

(54) Title: SYNTHETIC HERBICIDE RESISTANCE GENE



(57) Abstract: The invention provides a DNA molecule, a DNA construct, a transgenic plant and a transgenic plant part comprising a synthetic DNA sequence. The synthetic DNA sequence encodes an enzyme that degrades 2,4-dichlorophenoxyacetic acid (2,4-D) to dichlorophenol. The synthetic DNA sequence comprises a natural microbial sequence that encodes the enzyme in which at least a plurality of the codons of the natural microbial sequence have been replaced by codons more preferred by a plant. The invention also provides a method of controlling weeds in a field containing transgenic plants according to the invention by applying an auxin herbicide, such as 2,4-D, to the field. The

invention further provides methods of selecting plants and plant cells that have been transformed with a DNA construct according to the invention using an auxin herbicide.

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SYNTHETIC HERBICIDE RESISTANCE GENE

FIELD OF THE INVENTION

The present invention relates to a synthetic herbicide-resistance gene, its use to prepare herbicide-resistant transgenic plants and its use as a selection marker.

BACKGROUND OF THE INVENTION

2,4-Dichlorophenoxyacetic acid (2,4-D) is a herbicide used to control broadleaf weeds. 2,4-D is degraded by *Alcaligenes eutrophus* and other microorganisms. The gene which encodes the first enzyme in the *A. eutrophus* 2,4-D degradation pathway is *tfdA*. This gene encodes a dioxygenase which catalyzes the conversion of 2,4-D to 2,4-dichlorophenol (DCP). DCP is much less toxic to plants than 2,4-D, and transgenic tobacco plants, cotton plants, and hardwood trees containing the *tfdA* gene have been reported to have increased tolerance to 2,4-D. Streber et al., *Bio/Technology*, **7**, 811-816 (1989); Lyon et al., *Plant Molec. Biol.*, **13**, 533-540 (1989); Bayley et al., *Theor. Appl. Genet.*, **83**, 645-649 (1992); Llewellyn and Last, in *Herbicide-Resistant Crops*, Chapter 10, pages 159-174 (Duke, ed., CRC Press (1996)); Last and Llewellyn, *Weed Science*, **47**, 401-404 (1999); U.S. Patents Nos. 6,153,401, 6,100,446, and 5,608,147; and PCT applications WO 98/38294 and WO 95/18862. However, transgenic plants resistant to levels of 2,4-D that might be encountered in agricultural situations have not been obtained. See Last and Llewellyn, *Weed Science*, **47**, 401-404 (1999). These authors suggest that codon optimization of the *tfdA* gene "might enhance tolerance levels." *Id.* at 404. The *tfdA* gene has also been used as a selection marker to identify transformed plants and plant cells. U.S. Patent No. 5,608,147; PCT application WO 95/18862.

SUMMARY OF THE INVENTION

The invention provides a DNA molecule comprising a synthetic DNA sequence. The synthetic DNA sequence encodes an enzyme that

degrades 2,4-dichlorophenoxyacetic acid to dichlorophenol. The synthetic DNA sequence comprises a natural microbial sequence that encodes the enzyme in which at least a plurality of the codons of the natural microbial sequence have been replaced by codons more preferred by a plant.

The invention also provides a DNA construct comprising the synthetic DNA sequence just described. In this construct, the synthetic DNA sequence is operatively linked to plant gene expression control sequences.

The invention further provides a transgenic plant or part of a plant. The transgenic plant or plant part comprises the synthetic DNA sequence operatively linked to plant gene expression control sequences.

The invention also provides a method of controlling weeds in a field containing transgenic plants according to the invention. The method comprises applying an amount of an auxin herbicide to the field effective to control the weeds in the field. The transgenic plants are tolerant to the auxin herbicide as a result of comprising and expressing the synthetic DNA sequence. Indeed, for the first time, transgenic plants have been produced which are tolerant to levels of auxin herbicides substantially greater than those normally used in agriculture for controlling weeds.

The invention further provides methods of selecting transformed plants and plant cells. The method of selecting transformed plant cells comprises providing a population of plant cells. At least some of the plant cells in the population are transformed with the DNA construct of the invention. Then, the resulting population of plant cells is grown in a culture medium containing an auxin herbicide at a concentration selected so that transformed plant cells proliferate and untransformed plant cells do not proliferate.

The method of selecting transformed plants comprises providing a population of plants suspected of comprising a transgenic plant according to the invention. Then, an auxin herbicide is applied to the population of plants, the amount of herbicide being selected so that transformed plants will grow and growth of untransformed plants will be inhibited.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1: Diagram of pProPClSV-SAD.

Figure 2: Diagram of pPZP211-PNPT-311g7.

Figure 3: Diagram of pPZP211-PNPT-512g7.

Figure 4: Diagram of pPZP211-PNPT-311-SAD.

Figure 5: Diagram of pPZP211-PNPT-512-SAD.

In these figures, SAD = 2,4-D-degrading synthetic gene adapted for dicots; CDS = coding sequence; AMV-Leader = 5' untranslated leader sequence from the 35S transcript of alfalfa mosaic virus; PClSV-Promoter = peanut chlorotic streak virus promoter; T-Left = T-DNA left border from *Agrobacterium tumefaciens* nopaline Ti plasmid pTiT37; 35SPolyA = 3' polyadenylation (polyA) termination signal sequence from the cauliflower mosaic virus (CaMV) 35S transcript; NPTII = neomycin phosphotransferase II; g7PolyA = 3' polyA termination signal from gene 7 within the T-Left border of an *A. tumefaciens* octopine plasmid; MCS = multiple cloning site; T-Right = T-DNA right border from *A. tumefaciens* Ti plasmid pTiT37.

DETAILED DESCRIPTION OF THE PRESENTLY-PREFERRED EMBODIMENTS OF THE INVENTION

The invention provides a synthetic DNA sequence. "Synthetic" is used herein to mean that the DNA sequence is not a naturally-occurring sequence.

The synthetic DNA sequence of the invention encodes an enzyme that degrades 2,4-dichlorophenoxyacetic acid (2,4-D) to dichlorophenol (DCP). The synthetic DNA sequence comprises a natural microbial

sequence that encodes the enzyme, in which at least a plurality of the codons of the natural microbial sequence have been replaced by codons more preferred by a plant.

A "natural microbial sequence" is the coding sequence of a naturally-occurring microbial gene that encodes an enzyme that can degrade 2,4-D to DCP. Thus, the "natural microbial sequence" may be the coding sequence of a cDNA or genomic clone isolated from a microorganism, may be a chemically-synthesized DNA molecule having the same coding sequence as that of such a clone, or may be a combination of such sequences.

Multi-enzyme pathways for 2,4-D degradation have been demonstrated in several genera of bacteria. See, e.g., Lyon et al., *Plant Molec. Biol.*, **13**, 533-540 (1989), and references cited therein. Strains of *Alcaligenes eutrophus* have been the most extensively studied of these bacteria. The first enzyme in the *A. eutrophus* degradation pathway converts 2,4-D to DCP. This enzyme, which is often referred to as a monooxygenase, but which is now known to be a dioxygenase (see Fukumori et al., *J. Bacteriol.*, **175**, 2083-2086 (1993)), is encoded by the *tfdA* gene. Thus, the natural microbial sequence may be the coding sequence of a cDNA or genomic clone encoding a *tfdA* dioxygenase. Such clones and their isolation are described in Bayley et al., *Theor. Appl. Genet.*, **83**, 645-649 (1992), Lyon et al., *Plant Molec. Biol.*, **13**, 533-540 (1989), Streber et al., *J. Bacteriology*, **169**, 2950-2955 (1987), Perkins and Lurquin, *J. Bacteriology*, **170**, 5669-5672 (1988), and U.S. Patents Nos. 6,100,446 and 6,153,401. See also Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1982), Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1989).

It is known that many bacteria are capable of degrading 2,4-D, including strains of *Acinetobacter*, *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Corynebacterium*, *Flavobacterium*, *Pseudomona* and

strains of Actinomycetes (e.g., *Nocardia* spp. and *Streptomyces viridochromogenes*) (see, e.g., Llewellyn and Last, in *Herbicide-Resistant Crops*, Chapter 10 (Stephen O. Duke ed., CRC Press Inc. (1996)), Bayley et al., *Theor. Appl. Genet.*, **83**, 645-649 (1992), Lyon et al., *Plant Molec. Biol.*, **13**, 533-540 (1989), and Streber et al., *J. Bacteriology*, **169**, 2950-2955 (1987), Loos, in *Degradation Of Herbicides*, pages 1-49 (Kearney and Kaufman, eds., Marcel Dekker, Inc., New York 1969), and references cited in these references), and additional strains of bacteria that degrade 2,4-D can be isolated by methods well known in the art (e.g., by isolation from soils where 2,4-D is used by the enrichment culture technique) (see, e.g., Loos, in *Degradation Of Herbicides*, pages 1-49 (Kearney and Kaufman, eds., Marcel Dekker, Inc., New York 1969)). Additional cDNA and genomic clones encoding an enzyme which converts 2,4-D to DCP can be obtained from these other bacteria in a similar manner as for the *tfdA* clones. See, e.g., Bayley et al., *Theor. Appl. Genet.*, **83**, 645-649 (1992); Lyon et al., *Plant Molec. Biol.*, **13**, 533-540 (1989); Streber et al., *J. Bacteriology*, **169**, 2950-2955 (1987); Perkins and Lurquin, *J. Bacteriology*, **170**, 5669-5672 (1988); U.S. Patents Nos. 6,100,446 and 6,153,401. See also Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1982), Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1989). In addition, or alternatively, isolated clones, portions of them, or sequences from them, can be used as probes to identify and isolate additional clones. See, e.g., Perkins and Lurquin, *J. Bacteriology*, **170**, 5669-5672 (1988); Bayley et al., *Theor. Appl. Genet.*, **83**, 645-649 (1992); U.S. Patents Nos. 6,100,446 and 6,153,401. See also Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1982), Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1989). The natural microbial sequence may be the coding sequence of one of these cDNA or genomic clones.

It is also known that yeasts and fungi are capable of degrading 2,4-D (see, e.g., Llewellyn and Last, in *Herbicide-Resistant Crops*, Chapter 10 (Stephen O. Duke ed., CRC Press Inc. (1996)); Han and New, *Soil Biol. Biochem.*, **26**, 1689-1695 (1994); Donnelly et al., *Applied And Environmental Microbiology*, **59**, 2642-2647 (1993); Loos, in *Degradation Of Herbicides*, pages 1-49 (Kearney and Kaufman, eds., Marcel Dekker, Inc., New York 1969), and references cited in these references), and additional strains of yeast and fungi that degrade 2,4-D can be obtained by methods well known in the art (e.g., by isolation from soils where 2,4-D is used by the enrichment culture technique) (see, e.g., Loos, in *Degradation Of Herbicides*, pages 1-49 (Kearney and Kaufman, eds., Marcel Dekker, Inc., New York 1969); Han and New, *Soil Biol. Biochem.*, **26**, 1689-1695 (1994)). Additional cDNA and genomic clones encoding an enzyme which converts 2,4-D to DCP can be obtained from yeast and fungi by methods well known in the art (see references cited above in the discussion of obtaining clones from bacteria), and the natural microbial sequence may be the coding sequence of one of these cDNA or genomic clones.

In addition, as noted above, the natural microbial sequence may be fully or partially chemically synthesized. To do so, a cDNA or genomic clone, obtained as described in the previous paragraphs, is sequenced by methods well known in the art. See, e.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1982), Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1989). A synthetic DNA sequence comprising the coding sequence of the cDNA or genomic clone can be fully or partially chemically synthesized using methods well known in the art. See, e.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1982), Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1989). For instance, DNA sequences may be synthesized by phosphoramidite chemistry in an automated DNA synthesizer. Also, the sequence of the *tfdA* gene from *A.*

eutrophus JMP134 is publically available (see Streber et al., *J. Bacteriology*, **169**, 2950-2955 (1987), U.S. Patents Nos. 6,100,446 and 6,153,401, and GenBank (accession number M16730)), and a synthetic DNA sequence comprising the coding sequence of the *A. eutrophus tfdA* gene can also be fully or partially chemically synthesized.

The preferred natural microbial sequence is a natural bacterial sequence. A "natural bacterial sequence" is the coding sequence of a naturally-occurring bacterial gene that encodes an enzyme that can degrade 2,4-D to DCP. Thus, the "natural bacterial sequence" may be the coding sequence of a cDNA or genomic clone isolated from a bacterium, may be a chemically-synthesized DNA molecule having the same coding sequence as that of such a clone, or may be a combination of such sequences. Most preferably the natural bacterial sequence is the coding sequence of a cDNA or genomic clone isolated from a strain of *A. eutrophus*, a chemically-synthesized DNA molecule having the same coding sequence as that of such a clone, or a combination of such sequences.

As noted above, at least a plurality of the codons of the natural microbial sequence will be replaced by codons more preferred by a plant (also referred to herein as "plant-preferred codons"). A "codon more preferred by a plant" or a "plant-preferred codon" is a codon which is used more frequently by a plant to encode a particular amino acid than is the microbial codon encoding that amino acid. Preferably, the plant-preferred codon is the codon used most frequently by the plant to encode the amino acid. The plant codon usage may be that of plants in general, a class of plants (e.g., dicotyledonous plants), a specific type of plant (e.g., cotton or soybeans), etc. The codon usage or preferences of a plant or plants can be deduced by methods known in the art. See, e.g., *Maximizing Gene Expression*, pages 225-85 (Reznikoff & Gold, eds., 1986), Perlak et al., *Proc. Natl. Acad. Sci. USA*, **88**,

3324-3328 (1991), PCT WO 97/31115, PCT WO 97/11086, EP 646643, EP 553494, and U.S. Patents Nos. 5,689,052, 5,567,862, 5,567,600, 5,552,299 and 5,017,692. For instance, the codons used by the plant or plants to encode all of the different amino acids in a selection of proteins expressed by the plant or plants, preferably those proteins which are highly expressed, are tabulated. This can be done manually or using software designed for this purpose (see PCT application WO 97/11086).

The use of codons more preferred by the plant in which the synthetic DNA sequence will be expressed will improve expression as compared to use of the natural microbial sequence. The published reports indicate that codon usage affects gene expression in plants at the level of mRNA stability and translational efficiency. See, e.g., Perlak et al., *Proc. Natl. Acad. Sci. USA*, **88**, 3324-3328 (1991); Adang et al., *Plant Molec. Biol.*, **21**:1131-1145 (1993); Sutton et al., *Transgenic Res.*, **1**:228-236 (1992). Not all of the codons of the natural microbial sequence need to be changed to plant-preferred codons in order to obtain improved expression. However, preferably at least the codons least preferred by the plant are changed to plant-preferred codons. "Codons least preferred by the plant" are those codons in the natural microbial sequence that are used least by the plant or plants in question to encode a particular amino acid. Preferably greater than about 50%, most preferably at least about 80%, of the microbial codons are changed to plant-preferred codons.

Plant-preferred codons can be introduced into the natural microbial sequence by methods well known in the art. For instance, site-directed mutagenesis can be used. See Perlak et al., *Proc. Natl. Acad. Sci. USA*, **88**, 3324-3328 (1991). See also Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1982), Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1989). However, the plant-

preferred codons are preferably introduced into the natural microbial sequence by chemically synthesizing the entire DNA sequence encoding the 2,4-D degrading enzyme. In particular, chemical synthesis is highly preferred when a large number of microbial codons are replaced by plant-preferred codons. In addition, chemical synthesis has a number of advantages. For instance, using chemical synthesis allows other changes to the sequence of the DNA molecule or its encoded protein to be made to, e.g., optimize expression (e.g., eliminate mRNA secondary structures that interfere with transcription or translation, eliminate undesired potential polyadenylation sequences, and alter the A+T and G+C content), add unique restriction sites at convenient points, delete protease cleavage sites, etc.

The synthetic DNA sequence having plant-preferred codons substituted for at least a plurality of microbial codons will encode the same amino acid sequence as the natural microbial sequence if these substitutions are the only differences in the sequence of the synthetic DNA sequence as compared to the natural microbial sequence. However, the synthetic DNA sequence may comprise additional changes as compared to the natural microbial sequence. For instance, the synthetic DNA sequence may encode an enzyme which degrades 2,4-D to DCP, but which has an altered amino acid sequence as compared to the enzyme encoded by the (unmutated) natural microbial sequence as a result of one or more substitutions, additions or deletions in the natural microbial sequence. Methods of making such substitutions, additions and deletions are well known in the art and are described above.

Assays for determining whether 2,4-D has been degraded to DCP are well known in the art. See, e.g., Streber et al., *J. Bacteriol.*, **169**, 2950-2955 (1987); Perkins et al., *J. Bacteriol.*, **170**, 5669-5672 (1988); Streber et al., *Bio/Technology*, **7**, 811-816 (1989); Lyon et al., *Plant Molec. Biol.*, **13**, 533-540 (1989); Bayley et al., *Theor. Appl. Genet.*, **83**, 645-649 (1992); Fukumori et al., *J.*

Bacteriol., **175**, 2083 (1993); Lyon et al., *Transgenic Res*, **2**, 162-169 (1993); Llewellyn and Last, in *Herbicide-Resistant Crops*, Chapter 10, pages 159-174 (Duke, ed., CRC Press (1996)); Last and Llewellyn, *Weed Science*, **47**, 401-404 (1999). Also, tolerance to 2,4-D and other auxin herbicides may be used to demonstrate this conversion. See below and references just cited.

The invention also provides DNA constructs comprising the synthetic DNA sequence operatively linked to plant gene expression control sequences. "DNA constructs" are defined herein to be constructed (non-naturally occurring) DNA molecules useful for introducing DNA into host cells, and the term includes chimeric genes, expression cassettes, and vectors.

As used herein "operatively linked" refers to the linking of DNA sequences (including the order of the sequences, the orientation of the sequences, and the relative spacing of the various sequences) in such a manner that the encoded protein is expressed. Methods of operatively linking expression control sequences to coding sequences are well known in the art. See, e.g., Maniatis et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1982), Sambrook et al., *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor, NY (1989).

"Expression control sequences" are DNA sequences involved in any way in the control of transcription or translation. Suitable expression control sequences and methods of making and using them are well known in the art.

The expression control sequences must include a promoter. The promoter may be any DNA sequence which shows transcriptional activity in the chosen plant cells, plant parts, or plants. The promoter may be inducible or constitutive. It may be naturally-occurring, may be composed of portions of various naturally-occurring promoters, or may be partially or totally synthetic.

Guidance for the design of promoters is provided by studies of promoter structure, such as that of Harley and Reynolds, *Nucleic Acids Res.*, **15**, 2343-61 (1987). Also, the location of the promoter relative to the transcription start may be optimized. See, e.g., Roberts, et al., *Proc. Natl Acad. Sci. USA*, **76**, 760-4 (1979). Many suitable promoters for use in plants are well known in the art.

For instance, suitable constitutive promoters for use in plants include: the promoters from plant viruses, such as the peanut chlorotic streak caulimovirus (PClSV) promoter (U.S. Patent No. 5,850,019), the 35S promoter from cauliflower mosaic virus (CaMV) (Odell et al., *Nature* **313**:810-812 (1985)), promoters of *Chlorella* virus methyltransferase genes (U.S. Patent No. 5,563,328), and the full-length transcript promoter from figwort mosaic virus (FMV) (U.S. Patent No. 5,378,619); the promoters from such genes as rice actin (McElroy et al., *Plant Cell* **2**:163-171 (1990)), ubiquitin (Christensen et al., *Plant Mol. Biol.* **12**:619-632 (1989) and Christensen et al., *Plant Mol. Biol.* **18**:675-689 (1992)), pEMU (Last et al., *Theor. Appl. Genet.* **81**:581-588 (1991)), MAS (Velten et al., *EMBO J.* **3**:2723-2730 (1984)), maize H3 histone (Lepetit et al., *Mol. Gen. Genet.* **231**:276-285 (1992) and Atanassova et al., *Plant Journal* **2**(3):291-300 (1992)), *Brassica napus* ALS3 (PCT application WO 97/41228); and promoters of various *Agrobacterium* genes (see U.S. Patents Nos. 4,771,002, 5,102,796, 5,182,200, 5,428,147).

Suitable inducible promoters for use in plants include: the promoter from the ACE1 system which responds to copper (Mett et al. *PNAS* **90**:4567-4571 (1993)); the promoter of the maize In2 gene which responds to benzenesulfonamide herbicide safeners (Hershey et al., *Mol. Gen. Genetics* **227**:229-237 (1991) and Gatz et al., *Mol. Gen. Genetics* **243**:32-38 (1994)), and the promoter of the Tet repressor from Tn10 (Gatz et al., *Mol. Gen. Genet.* **227**:229-237

(1991). A particularly preferred inducible promoter for use in plants is one that responds to an inducing agent to which plants do not normally respond. An exemplary inducible promoter of this type is the inducible promoter from a steroid hormone gene, the transcriptional activity of which is induced by a glucocorticosteroid hormone (Schena et al., *Proc. Natl. Acad. Sci. USA* **88**:10421 (1991)) or the recent application of a chimeric transcription activator, XVE, for use in an estrogen receptor-based inducible plant expression system activated by estradiol (Zuo et al., *The Plant Journal*, **24**:265-273 (2000)). Other inducible promoters for use in plants are described in EP 332104, PCT WO 93/21334 and PCT WO 97/06269.

Finally, promoters composed of portions of other promoters and partially or totally synthetic promoters can be used. See, e.g., Ni et al., *Plant J.*, **7**:661-676 (1995) and PCT WO 95/14098 describing such promoters for use in plants.

The promoter may include, or be modified to include, one or more enhancer elements. Preferably, the promoter will include a plurality of enhancer elements. Promoters containing enhancer elements provide for higher levels of transcription as compared to promoters that do not include them. Suitable enhancer elements for use in plants include the PClSV enhancer element (U.S. Patent No. 5,850,019), the CaMV 35S enhancer element (U.S. Patents Nos. 5,106,739 and 5,164,316) and the FMV enhancer element (Maiti et al., *Transgenic Res.*, **6**, 143-156 (1997)). See also PCT WO 96/23898 and *Enhancers And Eukaryotic Expression* (Cold Spring Harbor Press, Cold Spring Harbor, NY, 1983).

For efficient expression, the coding sequences are preferably also operatively linked to a 3' untranslated sequence. The 3' untranslated sequence will include a transcription termination sequence and a polyadenylation sequence. The 3' untranslated region can be obtained from the flanking regions of genes from

Agrobacterium, plant viruses, plants or other eukaryotes. Suitable 3' untranslated sequences for use in plants include those of the cauliflower mosaic virus 35S gene, the phaseolin seed storage protein gene, the pea ribulose biphosphate carboxylase small subunit E9 gene, the soybean 7S storage protein genes, the octopine synthase gene, and the nopaline synthase gene.

A 5' untranslated sequence is also employed. The 5' untranslated sequence is the portion of an mRNA which extends from the 5' CAP site to the translation initiation codon. This region of the mRNA is necessary for translation initiation in plants and plays a role in the regulation of gene expression. Suitable 5' untranslated regions for use in plants include those of alfalfa mosaic virus, cucumber mosaic virus coat protein gene, and tobacco mosaic virus.

As noted above, the DNA construct may be a vector. The vector may contain one or more replication systems which allow it to replicate in host cells. Self-replicating vectors include plasmids, cosmids and viral vectors. Alternatively, the vector may be an integrating vector which allows the integration into the host cell's chromosome of the synthetic DNA sequence encoding the 2,4-D-degrading enzyme. The vector desirably also has unique restriction sites for the insertion of DNA sequences. If a vector does not have unique restriction sites, it may be modified to introduce or eliminate restriction sites to make it more suitable for further manipulations.

The DNA constructs of the invention can be used to transform any type of plant cells (see below). A genetic marker must be used for selecting transformed plant cells ("a selection marker"). Selection markers typically allow transformed cells to be recovered by negative selection (*i.e.*, inhibiting growth of cells that do not contain the selection marker) or by screening for a product encoded by the selection marker.

The most commonly used selectable marker gene for plant transformation is the neomycin phosphotransferase II (*nptII*) gene, isolated from Tn5, which, when placed under the control of plant expression control signals, confers resistance to kanamycin. Fraley et al., *Proc. Natl. Acad. Sci. USA*, **80**:4803 (1983). Another commonly used selectable marker gene is the hygromycin phosphotransferase gene which confers resistance to the antibiotic hygromycin. Vanden Elzen et al., *Plant Mol. Biol.*, **5**:299 (1985).

Additional selectable marker genes of bacterial origin that confer resistance to antibiotics include gentamycin acetyl transferase, streptomycin phosphotransferase, aminoglycoside-3'-adenyl transferase, and the bleomycin resistance determinant. Hayford et al., *Plant Physiol.* **86**:1216 (1988), Jones et al., *Mol. Gen. Genet.* **210**:86 (1987), Svab et al., *Plant Mol. Biol.* **14**:197 (1990), Hille et al., *Plant Mol. Biol.* **7**:171 (1986). Other selectable marker genes confer resistance to herbicides such as glyphosate, glufosinate or bromoxynil. Comai et al., *Nature* **317**:741-744 (1985), Stalker et al., *Science* **242**:419-423 (1988), Hinchey et al., *Bio/Technology* **6**:915-922 (1988), Stalker et al., *J. Biol. Chem.* **263**:6310-6314 (1988), and Gordon-Kamm et al., *Plant Cell* **2**:603-618 (1990).

Other selectable marker genes for plant transformation are not of bacterial origin. These genes include, for example, mouse dihydrofolate reductase, plant 5-enolpyruvylshikimate-3-phosphate synthase, and plant acetolactate synthase. Eichholtz et al., *Somatic Cell Mol. Genet.* **13**:67 (1987), Shah et al., *Science* **233**:478 (1986), Charest et al., *Plant Cell Rep.* **8**:643 (1990), EP 154,204.

Commonly used genes for screening presumptively transformed cells include β -glucuronidase (GUS), β -galactosidase, luciferase, and chloramphenicol acetyltransferase. Jefferson, R.A., *Plant Mol.*

Biol. Rep. 5:387 (1987)., Teeri et al., *EMBO J.* 8:343 (1989), Koncz et al., *Proc. Natl. Acad. Sci. USA* 84:131 (1987), De Block et al., *EMBO J.* 3:1681 (1984), green fluorescent protein (GFP) (Chalfie et al., *Science* 263:802 (1994), Haseloff et al., *TIG* 11:328-329 (1995) and PCT application WO 97/41228). Another approach to the identification of relatively rare transformation events has been use of a gene that encodes a dominant constitutive regulator of the *Zea mays* anthocyanin pigmentation pathway. Ludwig et al., *Science* 247:449 (1990).

According to another aspect of the present invention, tolerance to an auxin herbicide can be used as a selection marker for plants and plant cells. "Auxin herbicide" is used herein to refer to phenoxy auxins (phenoxy herbicides), which include 2,4-D, 4-chlorophenoxyacetic acid, 4,-chloro-2-methylphenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 2,4-dichlorophenoxybutyric acid, 4-(2-methyl-4-chlorophenoxy)butyric acid, 2-(4-chlorophenoxy)propionic acid, 2-(2,4-dichlorophenoxy)propionic acid, 2-(2,4,5-trichlorophenoxy)propionic acid, and salts (including amine salts) and esters of these acids. Auxin herbicides are available commercially. See *Crop Protection Reference* (Chemical & Pharmaceutical Press, Inc., New York, NY, 11th ed., 1995). The preferred auxin herbicides are 2,4-D and its salts (including amine salts) and esters. "Tolerance" means that transformed plant cells are able to grow (survive, proliferate and regenerate into plants) when placed in culture medium containing a level of an auxin herbicide that prevents untransformed cells from doing so. "Tolerance" also means that transformed plants are able to grow after application of an amount of an auxin herbicide that inhibits the growth of untransformed plants.

Methods of selecting transformed plant cells are well known in the art. Briefly, at least some of the plant cells in a population of plant cells (e.g., an explant or an embryonic suspension culture) are transformed with a DNA construct comprising the

synthetic DNA sequence of the invention. The resulting population of plant cells is placed in culture medium containing an auxin herbicide at a concentration selected so that transformed plant cells will grow, whereas untransformed plant cells will not. Suitable concentrations of an auxin herbicide can be determined empirically as is known in the art. At least in the case of 2,4-D, this amount may further need to be an amount which inhibits adventitious shoot formation from untransformed plant cells and allows adventitious shoot formation from transformed plant cells, since this is apparently the case with the natural-occurring bacterial *tfdA* gene. See U.S. Patent No. 5,608,147 and PCT application WO 95/18862. In general, 2,4-D should be present in an amount ranging from about 0.001 mg/l to about 5 mg/l culture medium, preferably from about 0.01 mg/l to 0.2 mg/l culture medium.

Methods of selecting transformed plants are also known in the art. Briefly, an auxin herbicide is applied to a population of plants which may comprise one or more transgenic plants comprising a DNA construct of the invention providing for 2,4-D degradation. The amount of the auxin herbicide is selected so that transformed plants will grow, and the growth of untransformed plants will be inhibited. The level of inhibition must be sufficient so that transformed and untransformed plants can be readily distinguished (*i.e.*, inhibition must be statistically significant). Such amounts can be determined empirically as is known in the art. See also *Crop Protection Reference* (Chemical & Pharmaceutical Press, Inc., New York, NY, 11th ed., 1995).

Selection based on tolerance to an auxin herbicide can be used in the production of plants tolerant to 2,4-D and other auxin herbicides, in which case the use of another selection marker may not be necessary. Absence of a separate selection marker is advantageous since it minimizes the number of foreign genes expressed.

Selection based on tolerance to an auxin herbicide can also be used in the production of transgenic plants that express other genes of interest. Many such genes are known and include genes coding for proteins of commercial value and genes that confer improved agronomic traits on plants (see, e.g., PCT WO 97/41228, the complete disclosure of which is incorporated herein by reference).

The DNA constructs of the invention can be used to transform a variety of plant cells. The synthetic DNA sequence coding for the 2,4-D-degrading enzyme and the selection marker, if a separate selection marker is used, may be on the same or different DNA constructs. Preferably, they are arranged on a single DNA construct as a transcription unit so that all of the coding sequences are expressed together. Also, the gene(s) of interest and the synthetic DNA sequence coding for the 2,4-D-degrading enzyme, when tolerance to an auxin herbicide is being used as a selection marker, may be on the same or different DNA constructs. Such constructs are prepared in the same manner as described above.

Suitable host cells include plant cells of any kind (see below). Preferably, the plant cell is one that does not normally degrade auxin herbicides. However, the present invention can also be used to increase the level of degradation of auxin herbicides in plants that normally degrade such herbicides.

Thus, the "transgenic" plants, plant parts, and plant cells of the invention include plants, plant parts and plant cells that do not normally degrade auxin herbicides, but which have been transformed according to the invention so that they are able to degrade these herbicides, and progeny of such transformed plants, plant parts and plant cells. The "transgenic" plants, plant parts and plant cells of the invention also include plants, plant parts and plant

cells that normally degrade auxin herbicides, but which have been transformed according to the invention so that they are able to degrade more of these herbicides or to degrade them more efficiently, and progeny of such transformed plants, plant parts and plant cells.

"Plant" should be understood as referring to a unicellular organism or a multicellular differentiated organism capable of photosynthesis, including algae, angiosperms (monocots and dicots), gymnosperms, bryophytes, ferns and fern allies. "Plant parts" are parts of multicellular differentiated plants and include seeds, pollen, embryos, flowers, fruits, shoots, leaves, roots, stems, explants, etc. "Plant cell" should be understood as referring to the structural and physiological unit of multicellular plants. Thus, the term "plant cell" refers to any cell that is a plant or is part of, or derived from, a plant. Some examples of cells encompassed by the present invention include differentiated cells that are part of a living plant, differentiated cells in culture, undifferentiated cells in culture, and the cells of undifferentiated tissue such as callus or tumors.

Methods of transforming plant cells are well known in the art. For instance, numerous methods for plant transformation have been developed, including biological and physical transformation protocols. See, for example, Miki et al., "Procedures for Introducing Foreign DNA into Plants" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B.R. and Thompson, J.E. Eds. (CRC Press, Inc., Boca Raton, 1993) pp. 67-88. In addition, vectors and *in vitro* culture methods for plant cell or tissue transformation and regeneration of plants are available. See, for example, Gruber et al., "Vectors for Plant Transformation" in *Methods in Plant Molecular Biology and Biotechnology*, Glick, B.R.

and Thompson, J.E. Eds. (CRC Press, Inc., Boca Raton, 1993) pp. 89-119.

The most widely utilized mechanism for introducing an expression vector into plants is based on the natural transformation systems of *Agrobacterium*. *A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, carry genes responsible for genetic transformation of the plant. See, for example, Kado, C.I., *Crit. Rev. Plant. Sci.* **10**:1 (1991). Descriptions of *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided by numerous references. See, for example, Horsch et al., *Science* **227**:1229 (1985), Hoekema et al., *Nature* **303**:179 (1983), de Framond et al., *Bio/Technology* **1**:262 (1983), Jordan et al., *Plant Cell Reports* **7**:281-284 (1988), Leple et al., *Plant Cell Reports* **11**:137-141 (1992), Stomp et al., *Plant Physiol.* **92**:1226-1232 (1990), Knauf et al., *Plasmid* **8**:45-54 (1982)), Gruber et al., supra, Miki et al., supra, Moloney et al., *Plant Cell Reports* **8**:238 (1989), PCT applications WO84/02913, WO84/02919 and WO84/02920, EP 116,718, and U.S. Patents Nos. 4,940,838, 5,464,763, and 5,929,300.

A generally applicable method of plant transformation is microprojectile-mediated transformation wherein DNA is carried on the surface of microprojectiles. The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds sufficient to penetrate plant cell walls and membranes. Sanford et al., *Part. Sci. Technol.* **5**:27 (1987), Sanford, J.C., *Trends Biotech.* **6**:299 (1988), Sanford, J.C., *Physiol. Plant* **79**:206 (1990), Klein et al., *Biotechnology* **10**:268 (1992), Klein et al., *Nature* **327**:70-73 (1987).

Another method for physical delivery of DNA to plants is sonication of target cells. Zhang et al., *Bio/Technology* 9:996 (1991). Alternatively, liposome or spheroplast fusion have been used to introduce expression vectors into plants. Deshayes et al., *EMBO J.*, 4:2731 (1985), Christou et al., *Proc Natl. Acad. Sci. USA* 84:3962 (1987). Direct uptake of DNA into protoplasts using CaCl_2 precipitation, polyvinyl alcohol or poly-L-ornithine have also been reported. Hain et al., *Mol. Gen. Genet.* 199:161 (1985) and Draper et al., *Plant Cell Physiol.* 23:451 (1982). Electroporation of protoplasts and whole cells and tissues have also been described. Donn et al., In Abstracts of VIIth International Congress on Plant Cell and Tissue Culture IAPTC, A2-38, p. 53 (1990); D'Halluin et al., *Plant Cell* 4:1495-1505 (1992), Spencer et al., *Plant Mol. Biol.* 24:51-61 (1994), and Fromm et al., *Proc. Natl. Acad. Sci. USA* 82:5824 (1985). Other techniques include microinjection (Crossway, *Mol. Gen. Genetics*, 202:179-185 (1985)), polyethylene glycol transformation (Krens et al., *Nature* 296:72-74 (1982)), fusion of protoplasts with other entities, either minicells, cells, lysosomes, or other fusible lipid-surfaced bodies (Fraley et al., *Proc. Natl. Acad. Sci. USA* 79:1859-1863 (1982)), and techniques set forth in U.S. Pat. No. 5,231,019).

After selection, transformed plant cells are regenerated into transgenic plants. Plant regeneration techniques are well known in the art and include those set forth in the *Handbook of Plant Cell Culture*, Volumes 1-3, Evans et al., eds. Macmillan Publishing Co., New York, N.Y. (1983, 1984, 1984, respectively); Predieri and Malavasi, *Plant Cell, Tissue, and Organ Culture* 17:133-142 (1989); James, D. J., et al., *J. Plant Physiol.* 132:148-154 (1988); Fasolo, F., et al., *Plant Cell, Tissue, and Organ Culture* 16:75-87 (1989); Valobra and James, *Plant Cell, Tissue, and Organ Culture* 21:51-54 (1990); Srivastava, P.S., et al., *Plant Science* 42:209-214 (1985); Rowland and Ogden, *Hort. Science* 27:1127-1129 (1992);

Park and Son, *Plant Cell, Tissue, and Organ Culture* 15:95-105 (1988); Noh and Minocha, *Plant Cell Reports* 5:464-467 (1986); Brand and Lineberger, *Plant Science* 57:173-179 (1988); Bozhkov, P.V. et al., *Plant Cell Reports* 11:386-389 (1992); Kvaalen and von Arnold, *Plant Cell, Tissue, and Organ Culture* 27:49-57 (1991); Tremblay and Tremblay, *Plant Cell Tissue, and Organ Culture* 27:95-103 (1991); Gupta and Pullman, U.S. Pat. No. 5,036,007; Michler and Bauer, *Plant Science* 77:111-118 (1991); Wetzstein, H.Y., et al., *Plant Science* 64:193-201 (1989); McGranahan, G.H., et al., *Bio/Technology* 6:800-804 (1988); Gingas, V.M., *Hort. Science* 26:1217-1218 (1991); Chalupa, V., *Plant Cell Reports* 9:398-401 (1990); Gingas and Lineberger, *Plant Cell, Tissue, and Organ Culture* 17:191-203 (1989); Bureno, M.A., et al., *Phys. Plant.* 85:30-34 (1992); and Roberts, D.R., et al., *Can. J. Bot.* 68:1086-1090 (1990).

Transgenic plants of any type may be produced according to the invention. Such plants include, for example, species from the genera *Fragaria*, *Lotus*, *Medicago*, *Onobrychis*, *Trifolium*, *Trigonella*, *Vigna*, *Citrus*, *Linum*, *Ceranium*, *Manihot*, *Daucus*, *Arabidopsis*, *Brassica*, *Raphanus*, *Sinapis*, *Atropa*, *Capsicum*, *Datura*, *Hyoscyamus*, *Lycopersicon*, *Nicotiana*, *Solanum*, *Petunia*, *Digitalis*, *Majorana*, *Cichorium*, *Helianthus*, *Lactuca*, *Bromus*, *Asparagus*, *Antirrhinum*, *Hererocallis*, *Nemesia*, *Pelargonium*, *Panicum*, *Pennisetum*, *Ranunculus*, *Sencia*, *Salpiglossis*, *Cucumis*, *Browalia*, *Glycine*, *Lolium*, *Zea*, *Triticum*, *Sorghum*, *Malus*, *Apium*, *Datura* and woody dicotyledonous forest tree species. In particular, broadleaf plants (including beans, soybeans, cotton, peas, potatoes, sunflowers, tomatoes, tobacco, fruit trees, ornamental plants and trees) that are currently known to be injured by auxin herbicides can be transformed so that they become tolerant to these herbicides. Other plants (such as corn, sorghum, small grains, sugarcane, asparagus, and grass) which are

currently considered tolerant to auxin herbicides can be transformed to increase their tolerance to these herbicides.

In yet another embodiment, the invention provides a method of controlling weeds in a field where transgenic plants are growing. The method comprises applying an effective amount of an auxin herbicide to the field to control the weeds. Methods of applying auxin herbicides and the amounts of them effective to control various types of weeds are known. See *Crop Protection Reference* (Chemical & Pharmaceutical Press, Inc., New York, NY, 11th ed., 1995). For the first time, as a result of the present invention, transgenic plants have been produced which are tolerant to levels of auxin herbicides substantially greater than those normally used in agriculture for controlling weeds.

EXAMPLES

EXAMPLE 1: Generation Of Synthetic Plant-Optimized Sequence Encoding A 2,4-D Dioxygenase

The DNA sequence of a 2,4-D dioxygenase (also often referred to as a monooxygenase; see above) gene isolated from *Alcaligenes eutrophus* was obtained from the sequence database GenBank (accession number M16730). From this DNA sequence, the amino acid sequence of the protein coded for by the single open-reading frame (ORF) was determined [SEQ ID NO:1]. A codon usage table reflecting dicotyledonous ORFs was derived from a composite selection of random cDNA sequences from cotton, *Arabidopsis* and tobacco extracted from the GenBank database. A codon usage table reflecting monocotyledonous ORFs was derived from a random selection of cDNA sequences from maize, also extracted from the GenBank database. These are Tables 1 and 2 below. Using these plant-specific codon usage tables, the derived primary amino acid sequence of the bacterial 2,4-D dioxygenase was converted into DNA coding sequences that reflected the codon preferences of dicotyledonous and monocotyledonous plants [SEQ ID NOS:2 and 3, respectively].

The synthetic plant-optimized 2,4-D dioxygenase ORFs [SEQ ID NOS:2 and 3], both dicot and monocot, were then used to design 2,4-D dioxygenase genes capable of efficient expression in transgenic plants. These synthetic genes were designated as SAD (Synthetic gene Aadapted for Dicots) and SAM (Synthetic gene Aadapted for Monocots) for the dicot and monocot versions, respectively. In order to generate a translatable transcript once the gene had been constructed and inserted into a plant genome, a 5' untranslated leader sequence representing the 5' untranslated leader sequence from the 35S transcript of alfalfa mosaic virus (AMV; Gallie et al., Nucleic Acids Res., 15:8693-8711 (1987)) was incorporated into the design of the synthetic genes. In addition, a signature sequence, encoding Cys Ala Gly, was added to the 3' end of the synthetic coding regions for each version of the synthetic gene. Finally, for ease of cloning, the designed sequences included a HindIII-specific overhang at the 5' end and a SalI-specific overhang at the 3' end. The complete designed sequences for the synthetic portions of the SAD and SAM genes are SEQ ID NOS:4 and 5.

To construct the designed synthetic portions of the SAD and SAM genes, each sequence was dissected into overlapping oligonucleotides, twelve oligonucleotides for each of the two strands resulting in a total of twenty-four oligonucleotides for each DNA sequence. A complete list of the oligonucleotides used to construct the synthetic portions of the SAD and SAM genes is given in Tables 3A, 3B, 4A, and 4B below. The oligonucleotides were synthesized using standard phosphoramidite chemistry by Integrated DNA Technologies, Inc., Coralville, Iowa. The synthetic DNA molecules were assembled using a procedure based upon the protocol described by Sutton et al. 1995 published on the World Wide Web (www.epicentre.com) using Ampliligase™ thermostable ligase (Epicentre Technologies Inc., Madison, WI). Oligonucleotides were first phosphorylated using T4 polynucleotide

kinase (Invitrogen Life Technologies, Carlsbad, CA) as mixtures of upper and lower strand oligonucleotides for each synthetic DNA construct. Each mixture contained 10 pmoles of each oligonucleotide, 70 mM Tris/HCl pH 7.6, 10 mM MgCl₂, 5 mM dithiothreitol (DTT), 0.1 mM ATP, and 10 units of T4 polynucleotide kinase, for a total volume of 25 μ l. Phosphorylation was achieved by incubation of the mixtures at 37°C for 30 minutes, followed by a denaturing incubation at 70°C for 10 minutes. To anneal and ligate the oligonucleotides, each reaction mixture was retreated at 70°C for 10 minutes in a thermocycler and subsequently cooled to 65°C over a 10-minute period. To each mixture, 65 μ l of water, 10 μ l of 10X Ampliligase buffer (Epicentre Technologies), and 2 μ l of Ampliligase (5 units/ μ l) were added sequentially, and the temperature was reduced to 40°C over a three hour period.

At this stage, in order to improve the efficiency of cloning, the complete synthetic DNA sequences for SAD and SAM were recovered from their respective annealing/ligation reactions by polymerase chain reaction (PCR) in an MJ Research Inc. (Waltham, MA) Model PTC-100 Thermocycler using Amplitaq Gold™ DNA polymerase under conditions supplied by the manufacturer, Perkin Elmer Life Sciences (Boston, MA). The PCR primers used for the recovery of each sequence were AGATCCTTTTTTATTTTAAATTTCTTTC [SEQ ID NO:6], a 28mer representing the 5' end of the AMV leader sequence and used for both the SAD and SAM recovery PCR reactions, and CTCCAGCACACTAAACAACAGCGTC [SEQ ID NO:7], a 25mer specific for the 3' end of the SAD sequence, and CTCCAGCACACTACACCACC [SEQ ID NO:8], a 20mer specific for the 3' end of the SAM sequence. PCR fragments corresponding to the appropriate size of 918 bp were gel purified as described in Ausubel et al., *Current Protocols In Molecular Biology* (Green/Wiley Interscience, New York, 1989) and cloned between two XcmI restriction sites in pUCR19, a modified pUC19 vector designed for rapid cloning of PCR fragments using T overhangs generated by XcmI digestion (described in O'Mahony and

Oliver, *Plant Molecular Biology*, 39:809-821 (1999)) to generate the plasmids pUCRs_{syn}SAD and pUCRs_{syn}SAM. Once cloned into these vectors, the inserts were sequenced to verify the sequence integrity of the designed synthetic portions of the SAD and SAM genes. DNA sequencing was performed by use of a dRhodamine Terminator Cycle Sequencing kit (PE Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Sequence reactions were analyzed using a Perkin Elmer/ABI Prism 310 automated sequencer.

TABLE 1: Dicot Codon Usage

Amino Acid	Codon	% Usage	Amino Acid	Codon	% Usage
Alanine	GCU	45.10	Lysine	AAG	55.14
	GCA	25.41		AAA	44.86
	GCC	17.62	Methionine	AUG	100.00
	GCG	11.87	Phenylalanine	UUC	51.60
Arginine	AGA	32.71		UUU	48.40
	AGG	23.18	Proline	CCU	37.73
	CGU	18.53		CCA	34.72
	CGA	10.55		CCG	14.94
	CGG	7.70		CCC	12.61
	CGC	7.33	Serine	UCU	27.64
Asparagine	AAC	51.76		UCA	19.32
	AAU	48.24		AGU	15.84
Aspartic Acid	GAU	65.56		AGC	13.98
	GAC	34.44		UCC	13.50
Cysteine	UGU	56.16		UCG	9.72
	UGC	43.84	Threonine	ACU	36.71
Glutamic Acid	GAG	51.01		ACA	28.49
	GAA	48.99		ACC	21.89
Glutamine	CAA	54.26		ACG	12.91
	CAG	45.74	Tryptophan	UGG	100.00
Glycine	GGA	36.34	Tyrosine	UAC	52.04
	GGU	35.49		UAU	47.96
	GGC	14.14	Valine	GUU	40.71
	GGG	14.03		GUG	26.01
Histidine	CAU	57.39		GUC	20.56
	CAC	42.61		GUA	12.71
Isoleucine	AUU	43.44	Stop	UAA	41.50
	AUC	36.28		UGA	40.73
	AUA	20.28		UAG	17.77
Leucine	UUG	24.73			
	CUC	19.86			
	CUU	19.86			
	UUA	12.99			
	CAG	11.69			
	CUA	10.87			

TABLE 2: Monocot Codon Usage

Amino Acid	Codon	% Usage	Amino Acid	Codon	% Usage
Alanine	GCC	36.21	Lysine	AAG	79.42
	GCG	24.24		AAA	20.58
	GCU	23.10	Methionine	AUG	100.00
	GCA	16.45			
Arginine	AGG	27.35		UUU	29.32
	CGC	27.09	Proline	CCA	27.77
	CGG	15.18		CCG	27.06
	AGA	12.94		CCC	23.97
	CGU	11.41		CCU	21.20
	CGA	6.03	Serine	AGC	24.00
Asparagine	AAC	68.33		UCC	23.41
	AAU	31.67		UCU	15.47
Aspartic Acid	GAC	62.72		UCG	13.97
	GAU	37.28		UCA	13.59
Cysteine	UGC	73.56		AGU	9.57
	UGU	26.44	Threonine	ACC	40.33
Glutamic Acid	GAG	73.29		ACU	21.27
	GAA	26.71		ACG	20.39
Glutamine	CAG	58.31		ACA	18.00
	CAA	41.69	Tryptophan	UGG	100.00
Glycine	GGC	41.40	Tyrosine	UAC	72.14
	GGG	20.28		UAU	27.86
	GGU	20.25	Valine	GUG	37.35
	GGA	18.07		GUC	32.71
Histidine	CAC	63.83		GUU	22.11
	CAU	36.17		GUA	7.83
Isoleucine	AUC	57.82	Stop	UGA	44.57
	AUU	28.52		UAG	28.24
	AUA	136.5		UAA	27.18
Leucine	CUC	27.01			
	CUU	27.01			
	CUG	23.80			
	UUG	11.90			
	CUA	6.23			
	UUA	4.05			

TABLE 3A
Dicot Oligonucleotides: Sense Strand

AGCTAGATCCTTTTTATTTTAAATTTTCTTTCAAATACTTCCAG	[SEQ ID NO:13]
ATCCATGTCTGTTGTTGCTAACCCTTTGCATCCTTTGTTTCGCTGCTGGAG TTGAGGATATTGATCTCAGAGAAGCATTGG	[SEQ ID NO:14]
GTTCTACTGAGGTGAGAGAAATTGAGAGACTCATGGACGAAAAGTCAGTT CTCGTTTTTCAGAGGTCAACCACTCTCACAG	[SEQ ID NO:15]
GATCAACAGATTGCTTTTGCTAGGAATTTTGGACCTTTGGAGGGTGGATT CATCAAAGTGAACCAGAGACCATCTAGGTT	[SEQ ID NO:16]
CAAATATGCTGAACTCGCTGATATCTCTAATGTTTCATTGGATGGTAAGG TGGCACAAAGAGACGCTAGAGAAGTTGTGG	[SEQ ID NO:17]
GAAATTTTGCAAATCAATTGTGGCATTCTGATTCTTCATTCCAACAGCCA GCAGCTAGATATTCTATGTTGTCAGCTGTT	[SEQ ID NO:18]
GTTGTGCCTCCTTCTGGAGGTGATACAGAATTTTGTGATATGAGGGCAGC TTACGATGCTCTCCCAAGGGATTTCAGCTC	[SEQ ID NO:19]
TGAACTCGAGGGATTGAGAGCTGAACATTACGCTTTGAACTCAAGATTTCT TCTTGGGAGATACTGATTACTCAGAGGCAC	[SEQ ID NO:20]
AGAGAAACGCTATGCCTCCTGTAACTGGCCTCTCGTTAGGACTCATGCT GGTTCTGGTAGAAAGTTCTTGTTTATTGGA	[SEQ ID NO:21]
GCACATGCTTCACATGTTGAGGGTCTCCCTGTTGCTGAGGGAAGAATGTT GCTCGCTGAATTGCTCGAACATGCTACTCA	[SEQ ID NO:22]
AAGAGAGTTTGTGTTATAGACACAGATGGAATGTTGGTGAAGTTGGTTATGT GGGATAATAGATGTGTGTTGCATAGAGGTA	[SEQ ID NO:23]
GGAGATATGATATTTCTGCTAGAAGGGAAGTCTCAGAGGGCTACTACTTTG GATGACGCTGTTGTTTAGTGTGCTGGAG	[SEQ ID NO:24]

TABLE 3B
Dicot Oligonucleotides: Nonsense Strand

GAACAAAGGATGCAAAGGGTTAGCAACAACAGACATGGATCTGGAAGTAT TTGAAAGAAAATTAAAAATAAAAAGGATCT	[SEQ ID NO:25]
TCGTCCATGAGTCTCTCAATTTCTCTCACCTCAGTAGAACCCAATGCTTC TCTGAGATCAATATCCTCAACTCCAGCAGC	[SEQ ID NO:26]
CCAAAGGTCCAAAATTCCTAGCAAAAGCAATCTGTTGATCCTGTGAGAGT GGTTGACCTCTGAAAACGAGAACTGACTTT	[SEQ ID NO:27]
CAATGAAACATTAGAGATATCAGCGAGTTCAGCATATTTGAACCTAGATG GTCTCTGGTTCACTTTGATGAATCCACCTT	[SEQ ID NO:28]
AATGAAGAATCAGAATGCCACAATTGATTGCAAAATTTCCACAACCTTC TCTAGCGTCTCTTTGTGCCACCTTACCATC	[SEQ ID NO:29]
TATCACAAAATTCTGTATCACCTCCAGAAGGAGGCACAACAACAGCTGAC AACATAGAAATATCTAGCTGCTGGCTGTTGG	[SEQ ID NO:30]
GTTCAAAGCGTAATGTTTCAGCTCTCAATCCCTCGAGTTCAGACTGCAAAT CCCTTGGGAGAGCATCGTAAGCTGCCCTCA	[SEQ ID NO:31]
CTAACGAGAGGCCAGTTAACAGGAGGCATAGCGTTTCTCTGTGCCTCTGA GTAATCAGTATCTCCAAGAGAAATCTTGA	[SEQ ID NO:32]
CCTCAGCAACAGGGAGACCCTCAACATGTGAAGCATGTGCTCCAATAAAC AAGAACTTTCTACCAGAACCAGCATGAGTC	[SEQ ID NO:33]
GTCACCAACATTCCATCTGTGTCTATAAACAACTCTCTTTGAGTAGCAT GTTTCGAGCAATTCAGCGAGCAACATTCTTC	[SEQ ID NO:34]
GCCCTTCTGAGTTCCCTTCTAGCAGAAATATCATATCTCCTACCTCTATG CAACACACATCTATTATCCACATAACCAA	[SEQ ID NO:35]
TCGACTCCAGCACACTAAACAACAGCGTCATCCAAAGTAGTA	[SEQ ID NO:36]

TABLE 4A
Monocot oligonucleotides: Sense strand

AGCTAGATCCTTTTTATTTTAAATTTTCTTTCAAATACTTCCAG	[SEQ ID NO: 37]
ATCCATGTCCGTGGTGGCCAACCCACTCCACCCGCTCTTCGCGGCCGGCG TGGAGGATATCGACCTCAGGGAGGCGCTGG	[SEQ ID NO: 38]
GCAGCACCGAAGTGCGCGAAATCGAGAGGCTCATGGACGAGAAGAGCGTC CTCGTCTTCGCGGCCAACCCTCTCACAG	[SEQ ID NO: 39]
GATCAACAGATTGCTTTTGCTAGGAATTTTGGACCTTTGGAGGGTGGATT CATCAAGGTGAACCAGCGCCCGTCCAGGTT	[SEQ ID NO: 40]
CAAGTACGCTGAACTGGCCGACATCAGCAACGTGTCCCTCGATGGGAAGG TGGCCAGAGGGACGCTAGGGAAGTTGTGG	[SEQ ID NO: 41]
GCAACTTCGCCAACCAACTGTGGCACTCCGATAGCTCTTTCCAACAGCCA GCAGCCAGGTACTCCATGCTGAGCGCCGTC	[SEQ ID NO: 42]
GTCGTGCCACCATCCGGCGGTGACACCGAGTTCTGCGATATGCGCGCCGC GTACGACGCCCTCCCGAGGGATCTGCAGAG	[SEQ ID NO: 43]
CGAGCTGGAGGGCCTCCGCGCGGAGCACTACGCCCTCAACAGCAGGTTCC TCCTGGGGGACACTGACTACTCCGAGGCCC	[SEQ ID NO: 44]
AGAGGAACGCGATGCCACCACTGAACTGGCCCCTCGTCCGCACCCACGCT GGCAGCGGCCGCAAGTTCCTGTTCATCGGG	[SEQ ID NO: 45]
GCCCATGCCTCCCATGTGGAGGGTCTCCCTGTGCGGAGGGCCGCATGCT CCTGGCCGAGCTCCTGGAGCACGCCACCCA	[SEQ ID NO: 46]
ACGCGAGTTCGTCTACCGCCACAGGTGGAATGTCGGCGACCTCGTCATGT GGGATAACCGCTGCGTGCTGCACCGCGGCA	[SEQ ID NO: 47]
GGCGCTACGATATCAGCGCGCGCAGGGAACCTCAGGCGCGCCACCACCTC GACGACGCGGTGGTGTAGTGTGCTGGAG	[SEQ ID NO: 48]

TABLE 4B
Monocot oligonucleotides: Nonsense strand

GAAGAGCGGGTGGAGTGGGTGGCCACCACGGACATGGATCTGGAAGTAT TTGAAAGAAAATTAAAAATAAAAAGGATCT	[SEQ ID NO:49]
TCGTCCATGAGCCTCTCGATTTTCGCGCACTTCGGTGCTGCCAGCGCCTC CCTGAGGTCGATATCCTCCACGCCGGCCGC	[SEQ ID NO:50]
CCAAAGGTCCAAAATTCCTAGCAAAAGCAATCTGTTGATCCTGTGAGAGT GGTTGGCCGCGGAAGACGAGGACGCTCTTC	[SEQ ID NO:51]
GAGGGACACGTTGCTGATGTGCGCCAGTTCAGCGTACTTGAACCTGGACG GGCGCTGGTTCACCTTGATGAATCCACCCT	[SEQ ID NO:52]
AAAGAGCTATCGGAGTGCCACAGTTGGTTGGCGAAGTTGCCACAACCTTC CCTAGCGTCCCTCTGGGCCACCTTCCCATC	[SEQ ID NO:53]
TATCGCAGAACTCGGTGTACCGCCGGATGGTGGCACGACGACGGCGCTC AGCATGGAGTACCTGGCTGCTGGCTGTTGG	[SEQ ID NO:54]
GTTGAGGGCGTAGTGCTCCGCGCGGAGGCCCTCCAGCTCGCTCTGCAGAT CCCTCGGGAGGGCGTCTGTACGCGGCGCGCA	[SEQ ID NO:55]
CGGACGAGGGGCCAGTTCACTGGTGGCATCGCGTTCCTCTGGGCCTCGGA GTAGTCAGTGTCCTCCAGGAGGAACCTGCT	[SEQ ID NO:56]
CCTCCGCGACAGGGAGACCCTCCACATGGGAGGCATGGGCCCCGATGAAC AGGAACTTGCGGCCGCTGCCAGCGTGGGTG	[SEQ ID NO:57]
GTCGCGGACATTCCACCTGTGGCGGTAGACGAACTCGCGTTGGGTGGCGT GCTCCAGGAGCTCGGCCAGGAGCATGCGGC	[SEQ ID NO:58]
GCGCGCCTGAGTTCCCTGCGCGCGCTGATATCGTAGCGCCTGCCGCGGTG CAGCACGCAGCGGTTATCCACATGACGAG	[SEQ ID NO:59]
TCGACTCCAGCACACTACACCACCGCGTCGTCGAGGGTGGTG	[SEQ ID NO:60]

EXAMPLE 2: Construction Of A Plant-Expressible SAD Gene.

For the generation of a complete and plant-competent SAD gene, the synthetic portions of the SAD gene contained in pUCrsynSAD were removed by first releasing the 5' end of the synthetic sequence by digestion with XbaI and filling in the overhang with DNA polymerase I (Klenow large fragment) followed by digestion with KpnI. This fragment was ligated into the plasmid pProPClSV, a pUC19 plasmid containing an enhanced Peanut Chlorotic Streak Virus (PClSV) promoter derived from pKLP36 (described by Maiti and Shepherd, *Biochem. Biophys. Res. Com.*, **244**:440-444 (1998)) by cutting first with NcoI, treating with DNA polymerase I (Klenow large fragment) to fill in the generated overhang, and

subsequently cutting with KpnI. This generated the plasmid pProPClSV-SAD within which the synthetic portion of the SAD gene, including the 5' AMV leader and 3' region coding for the Cys Ala Gly signature, is directly linked to the 3' end of the PClSV promoter (Figure 1). This plasmid served as the source for the PClSV-SAD construction for insertion into the binary vectors for final gene construction prior to introduction into *Agrobacterium* for plant transformation.

Two binary vectors were chosen for final SAD gene construction, pPZP211-PNPT-311g7 (Figure 2) and pPZP211-PNPT-512g7 (Figure 3). These two vectors are based on the pPZP family of vectors described by Hajdukiewicz et al., *Plant Molec. Biol.*, **25**:989-994 (1994) and are pPZP211 derivatives in which the neomycin phosphotransferase II (NPTII) gene for kanamycin resistance is driven by the PClSV promoter and a g7 polyA termination sequence is placed adjacent to a multicloning site (MCS, Figures 2 and 3). The only difference between these two vectors is the position of the MCS relative to the g7 polyA termination sequence. The g7 polyA termination sequence is the 3' polyA termination signal from gene 7 within the octopine T-Left region of an octopine *Agrobacterium tumefaciens* Ti plasmid and was isolated as an EcoRI-SalI fragment from pAP2034 (Velten and Schell, *Nucleic Acids*, **13**:6981-6998 (1985)).

The complete SAD gene was constructed by removal of the PClSV-SAD sequence from pProPClSV-SAD as a HindIII-SmaI fragment and insertion into both pPZP211-PNPT-311g7 and pPZP211-PNPT-512g7 that were first cut with BamHI, treated with DNA polymerase I (Klenow large fragment) to fill in the overhanging sequence, and subsequently digested with HindIII. These reactions generated the two vectors, pPZP211-PNPT-311-SAD (Figure 4) and pPZP211-PNPT-512-SAD (Figure 5), that contained the full plant expressible SAD gene in one of two orientations with respect to the PClSV-NPTII-35SpolyA construct. This design for insertion of the SAD gene

into plant genomes was implemented because of uncertainty as to the effect of having two PClSV promoter sequences in the same plasmid on both transformation and effective transmission of the expressed trait. By putting the SAD gene in the vectors such that the PClSV promoters were inserted as both direct and inverted repeats, the possibility of a negative outcome could be avoided.

After construction, the SAD genes in each vector were sequenced as described above to ensure fidelity. This sequencing revealed that, in the construction of pProPClSV-SAD, an out-of-frame ATG codon was introduced into the 5' untranslated leader sequence. The presence of this ATG codon could alter the translatability of the transcript that would be synthesized from the SAD gene and so was deleted by PCR mutagenesis to restore the normal AMV leader sequence. Following repair, the sequence was rechecked for fidelity. The original SAD gene containing the out-of-frame ATG was labelled SAD1 (since some transformation experiments had begun using this construct). The repaired SAD gene is referred to as SAD2 and is the only version of the gene used for integration of the SAD construct into the cotton genome.

EXAMPLE 3: Introduction Of SAD2 Into Cotton

The two binary vectors containing the SAD2 gene, pPZP211-PNPT-311-SAD2 and pPZP211-PNPT-512-SAD2, were individually introduced into the EHA 105 strain of *Agrobacterium tumefaciens* (Hood et al., *Transgenic Research*, 2:208-218 (1993)) by direct transformation as described by Walker-Peach and Velten, in *Plant Molecular Biology Manual*, section B1:1-19 (Gelvin, Shilperoort and Verma, eds., Kluwer Academic Publishers, Dordrecht, The Netherlands, 1994)). The constructs were subsequently introduced by *Agrobacterium* transfection into cotyledon explants from the cotton variety Coker 312 (Coker Seed Inc.). This was achieved by isolating sterile cotyledon tissue (derived from seedlings grown in culture from surface-sterilized seed as described by Trolinder and Gooden, *Plant Cell Reports*, 6:231-234 (1987)), generating

explants (by use of a sterile hole punch), and submerging the explants in a 48-hour-old culture of EHA 105, containing the appropriate construct, grown at 28°C. The explants were then transferred onto 2MST medium (MS medium + 0.2 mg/L 2,4-D and 0.1 mg/L kinetin) subsequent to removal of excess EHA 105. The infected cotyledon tissues were incubated on the 2MST medium for 2 days at 28°C prior to transfer to T1+KCL medium (MS medium + 0.1 mg/L 2,4-D and 0.1 mg/L kinetin + 50 mg/L kanamycin sulphate and 250 mg/L Cefotaxime). Once healthy callus tissue was formed, it was placed on fresh T1+KCL (with 0.05 mg/L 2,4-D) for a second round of selection. After six weeks, somatic embryos were generated from the surviving callus, and mature transgenic cotton plants were produced as described by Trolinder and Goodin, *Plant Cell Reports*, 6:231-234 (1987).

A total of 111 kanamycin-resistant cotton seedlings were generated (44 were generated in the pPZP211-PNPT-311-SAD2 transformations, and 67 in the pPZP211-PNPT-512-SAD2 transformations). Each plant was analyzed for the presence of the SAD synthetic coding sequence and the NPTII coding sequence by PCR to ensure the integrity of the inserted DNA. The PCR was performed as described above. The primers used for this analysis were GGAGTTGAGGATATTGATCTCAGAGAAGCATTG [SEQ ID NO:9] and GCGATCTGCTGATCCTGACTC [SEQ ID NO:10] for the SAD coding region and CGTCAAGAAGGCGATAGAAGG [SEQ ID NO:11] and GCTATGACTGGGCACAACAGAC [SEQ ID NO:12] for the NPTII coding region. Of the 44 pPZP211-PNPT-311-SAD seedlings that survived kanamycin treatment, 2 were shown to be negative by the PCR testing. Of the 67 pPZP211-PNPT-512-SAD seedlings that survived kanamycin treatment, 14 were negative in the PCR tests.

The remaining 95 plants were grown in pots in the greenhouse until at least the squaring stage (approximately 18" tall) and were then sprayed with 2,4-D amine (United Agri Products, Greeley, CO) at 1 lb/acre acid equivalents. This is twice the normal field rate

for 2,4-D applications. Of the 95 plants, 22 survived this treatment with little or no herbicide damage being evident. Eleven of these plants contained the pPZP211-PNPT-311-SAD2 construct, and 11 contained the pPZP211-PNPT-512-SAD2 construct. Each plant was grown to maturity in the greenhouse.

Of the 22 transgenic plants, only 7 produced seed. The remaining plants were apparently infertile. Presumably this infertility was an effect of the regeneration procedure, which is common for cotton. Of the seven fertile plants, 3 contained the pPZP211-PNPT-311-SAD2 construct, and 4 contained the pPZP211-PNPT-311-SAD2 construct.

To verify that the inserted synthetic SAD genes were inheritable and to gain an indication of the number of gene insertions, seeds from the seven fertile SAD transgenic cotton plants were planted into hydroponic rock wool slabs (Hummert, St. Louis, MO) that had been saturated with Peters Professional water-soluble fertilizer (5-11-26 HYDRO-SOL, supplemented with calcium nitrate and magnesium sulfate to provide a complete nutrient compliment; Hummert, St. Louis, MO). The hydroponic rock wool slabs were placed on benches in a greenhouse, and nutrients were maintained at optimal levels using a non-recycling hydroponic watering system. Plants were grown under greenhouse conditions ($28^{\circ}\text{C} \pm 5^{\circ}\text{C}$ air temperature) for 24 days. At this point, the plants were removed to a spray hood, sprayed with 2,4-D amine at the normal field rate of 1/2 lb/acre acid equivalents, maintained in the hood for 24 hours to allow the 2,4-D to volatilize, and then placed back in the greenhouse. The effect of the treatment was evaluated visually after 10-14 days, and the results are presented in Table 5 below.

TABLE 5

Transgenic Line	Number of plants	No Damage	Some symptoms	Severe damage	Ratio "Res"/Sens
311-22-1-4	43	16	16	11	3:1
311-22-1-5	47	6	18	23	1:1
311-22-1-8	56	14	28	14	3:1
512-21-1-5	56	8	34	14	3:1
512-21-4-3	30	7	20	3	9:1
512-21-4-5	41	5	24	12	2:1
512-24-4-4	63	7	21	35	1:1
2,4-D res control	55	7	33	15	3:1
2,4-D sens control	45	0	20	25	1:1

2,4-D res control = transgenic 2,4-D resistant cotton containing the naturally-occurring *tfdA* gene construct. (Bayley et al., Theoretical Applied Genetics, 83:645-649 (1992))

2,4-D sens control = Coker 312 (not transgenic) regenerated from somatic embryos in the same manner as those containing the SAD constructs

Some symptoms = some leaf wilt and minor leaf dessication

Severe damage = all leaves wilted and desiccation damage readily evident

The ratio "Res"/Sens was calculated as the number of plants that showed some resistance to 2,4-D treatment during the experiment divided by the combined number of plants that showed severe damage or death. The negative control of Coker 312 that had been regenerated from tissue culture did show some signs of resistance, so these ratios are not to be considered as definitive measures of Mendelian inheritance of the SAD gene. Nevertheless, all of the negative control plants did show 2,4-D-induced damage, whereas all of the transgenic lines that contain the SAD gene had individuals that exhibited no damage at all.

Five plants from each of the 7 lines that exhibited no damage when treated with 2,4-D 24 days after germination were chosen, and individual newly-formed leaf samples, one per plant, were taken for PCR testing, performed as described above. Each plant tested positive for the SAD construct by PCR. These plants were grown

for a further 14 days and then resprayed with 2,4-D amine at the normal field rate of 1/2 lb/acre acid equivalents. All 35 plants exhibited no damage following this treatment, whereas all negative controls did not survive this spray event. The 35 plants were grown to maturity, and seeds were collected.

The content of each of the references referred to hereinabove, including publications, patents, and published applications, are incorporated by reference herein.

SEQUENCE LISTING

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gag gat atc gac ctc agg gag gcg ctg ggc agc acc gaa gtg cgc gaa	96
Glu Asp Ile Asp Leu Arg Glu Ala Leu Gly Ser Thr Glu Val Arg Glu	
20 25 30	
atc gag agg ctc atg gac gag aag agc gtc ctc gtc ttc cgc ggc caa	144
Ile Glu Arg Leu Met Asp Glu Lys Ser Val Leu Val Phe Arg Gly Gln	
35 40 45	
cca ctc tca cag gat caa cag att gct ttt gct agg aat ttt gga cct	192
Pro Leu Ser Gln Asp Gln Gln Ile Ala Phe Ala Arg Asn Phe Gly Pro	
50 55 60	
ttg gag ggt gga ttc atc aag gtg aac cag cgc ccg tcc agg ttc aag	240
Leu Glu Gly Gly Phe Ile Lys Val Asn Gln Arg Pro Ser Arg Phe Lys	
65 70 75 80	
tac gct gaa ctg gcc gac atc agc aac gtg tcc ctc gat ggg aag gtg	288
Tyr Ala Glu Leu Ala Asp Ile Ser Asn Val Ser Leu Asp Gly Lys Val	
85 90 95	
gcc cag agg gac gct agg gaa gtt gtg ggc aac ttc gcc aac caa ctg	336
Ala Gln Arg Asp Ala Arg Glu Val Val Gly Asn Phe Ala Asn Gln Leu	
100 105 110	
tgg cac tcc gat agc tct ttc caa cag cca gca gcc agg tac tcc atg	384
Trp His Ser Asp Ser Ser Phe Gln Gln Pro Ala Ala Arg Tyr Ser Met	
115 120 125	
ctg agc gcc gtc gtc gtg cca cca tcc ggc ggt gac acc gag ttc tgc	432
Leu Ser Ala Val Val Val Pro Pro Ser Gly Gly Asp Thr Glu Phe Cys	
130 135 140	

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cgc agg gaa ctc agg cgc gcc acc acc ctc gac gac gcg gtg gtg tag 864
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43

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gct aac cct ttg cat cct ttg ttc gct gct gga gtt gag gat att gat      104
Ala Asn Pro Leu His Pro Leu Phe Ala Ala Gly Val Glu Asp Ile Asp
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ctc aga gaa gca ttg ggt tct act gag gtg aga gaa att gag aga ctc      152
Leu Arg Glu Ala Leu Gly Ser Thr Glu Val Arg Glu Ile Glu Arg Leu
              25              30              35

atg gac gaa aag tca gtt ctc gtt ttc aga ggt caa cca ctc tca cag      200
Met Asp Glu Lys Ser Val Leu Val Phe Arg Gly Gln Pro Leu Ser Gln
              40              45              50

gat caa cag att gct ttt gct agg aat ttt gga cct ttg gag ggt gga      248
Asp Gln Gln Ile Ala Phe Ala Arg Asn Phe Gly Pro Leu Glu Gly Gly
              55              60              65

ttc atc aaa gtg aac cag aga cca tct agg ttc aaa tat gct gaa ctc      296
Phe Ile Lys Val Asn Gln Arg Pro Ser Arg Phe Lys Tyr Ala Glu Leu
              70              75              80

gct gat atc tct aat gtt tca ttg gat ggt aag gtg gca caa aga gac      344
Ala Asp Ile Ser Asn Val Ser Leu Asp Gly Lys Val Ala Gln Arg Asp
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gct aga gaa gtt gtg gga aat ttt gca aat caa ttg tgg cat tct gat	392
Ala Arg Glu Val Val Gly Asn Phe Ala Asn Gln Leu Trp His Ser Asp	
105 110 115	
tct tca ttc caa cag cca gca gct aga tat tct atg ttg tca gct gtt	440
Ser Ser Phe Gln Gln Pro Ala Ala Arg Tyr Ser Met Leu Ser Ala Val	
120 125 130	
gtt gtg cct cct tct gga ggt gat aca gaa ttt tgt gat atg agg gca	488
Val Val Pro Pro Ser Gly Gly Asp Thr Glu Phe Cys Asp Met Arg Ala	
135 140 145	
gct tac gat gct ctc cca agg gat ttg cag tct gaa ctc gag gga ttg	536
Ala Tyr Asp Ala Leu Pro Arg Asp Leu Gln Ser Glu Leu Glu Gly Leu	
150 155 160	
aga gct gaa cat tac gct ttg aac tca aga ttt ctc ttg gga gat act	584
Arg Ala Glu His Tyr Ala Leu Asn Ser Arg Phe Leu Leu Gly Asp Thr	
165 170 175 180	
gat tac tca gag gca cag aga aac gct atg cct cct gtt aac tgg cct	632
Asp Tyr Ser Glu Ala Gln Arg Asn Ala Met Pro Pro Val Asn Trp Pro	
185 190 195	
ctc gtt agg act cat gct ggt tct ggt aga aag ttc ttg ttt att gga	680
Leu Val Arg Thr His Ala Gly Ser Gly Arg Lys Phe Leu Phe Ile Gly	
200 205 210	
gca cat gct tca cat gtt gag ggt ctc cct gtt gct gag gga aga atg	728
Ala His Ala Ser His Val Glu Gly Leu Pro Val Ala Glu Gly Arg Met	
215 220 225	
ttg ctc gct gaa ttg ctc gaa cat gct act caa aga gag ttt gtt tat	776
Leu Leu Ala Glu Leu Leu Glu His Ala Thr Gln Arg Glu Phe Val Tyr	
230 235 240	
aga cac aga tgg aat gtt ggt gac ttg gtt atg tgg gat aat aga tgt	824
Arg His Arg Trp Asn Val Gly Asp Leu Val Met Trp Asp Asn Arg Cys	
245 250 255 260	

gtg ttg cat aga ggt agg aga tat gat att tct gct aga agg gaa ctc 872
 Val Leu His Arg Gly Arg Arg Tyr Asp Ile Ser Ala Arg Arg Glu Leu
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 Met Ser Val Val
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 Ala Asn Pro Leu His Pro Leu Phe Ala Ala Gly Val Glu Asp Ile Asp
 5 10 15 20

ctc agg gag gcg ctg ggc agc acc gaa gtg cgc gaa atc gag agg ctc 152
 Leu Arg Glu Ala Leu Gly Ser Thr Glu Val Arg Glu Ile Glu Arg Leu
 25 30 35

atg gac gag aag agc gtc ctc gtc ttc cgc ggc caa cca ctc tca cag 200
 Met Asp Glu Lys Ser Val Leu Val Phe Arg Gly Gln Pro Leu Ser Gln
 40 45 50

gat caa cag att gct ttt gct agg aat ttt gga cct ttg gag ggt gga	248
Asp Gln Gln Ile Ala Phe Ala Arg Asn Phe Gly Pro Leu Glu Gly Gly	
55 60 65	
ttc atc aag gtg aac cag cgc ccg tcc agg ttc aag tac gct gaa ctg	296
Phe Ile Lys Val Asn Gln Arg Pro Ser Arg Phe Lys Tyr Ala Glu Leu	
70 75 80	
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Ala Asp Ile Ser Asn Val Ser Leu Asp Gly Lys Val Ala Gln Arg Asp	
85 90 95 100	
gct agg gaa gtt gtg ggc aac ttc gcc aac caa ctg tgg cac tcc gat	392
Ala Arg Glu Val Val Gly Asn Phe Ala Asn Gln Leu Trp His Ser Asp	
105 110 115	
agc tct ttc caa cag cca gca gcc agg tac tcc atg ctg agc gcc gtc	440
Ser Ser Phe Gln Gln Pro Ala Ala Arg Tyr Ser Met Leu Ser Ala Val	
120 125 130	
gtc gtg cca cca tcc ggc ggt gac acc gag ttc tgc gat atg cgc gcc	488
Val Val Pro Pro Ser Gly Gly Asp Thr Glu Phe Cys Asp Met Arg Ala	
135 140 145	
gcg tac gac gcc ctc ccg agg gat ctg cag agc gag ctg gag ggc ctc	536
Ala Tyr Asp Ala Leu Pro Arg Asp Leu Gln Ser Glu Leu Glu Gly Leu	
150 155 160	
cgc gcg gag cac tac gcc ctc aac agc agg ttc ctc ctg ggg gac act	584
Arg Ala Glu His Tyr Ala Leu Asn Ser Arg Phe Leu Leu Gly Asp Thr	
165 170 175 180	
gac tac tcc gag gcc cag agg aac gcg atg cca cca gtg aac tgg ccc	632
Asp Tyr Ser Glu Ala Gln Arg Asn Ala Met Pro Pro Val Asn Trp Pro	
185 190 195	
ctc gtc cgc acc cac gct ggc agc ggc cgc aag ttc ctg ttc atc ggg	680
Leu Val Arg Thr His Ala Gly Ser Gly Arg Lys Phe Leu Phe Ile Gly	
200 205 210	

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gcc cat gcc tcc cat gtg gag ggt ctc cct gtc gcg gag ggc cgc atg      728
Ala His Ala Ser His Val Glu Gly Leu Pro Val Ala Glu Gly Arg Met
      215                      220                      225

ctc ctg gcc gag ctc ctg gag cac gcc acc caa cgc gag ttc gtc tac      776
Leu Leu Ala Glu Leu Leu Glu His Ala Thr Gln Arg Glu Phe Val Tyr
      230                      235                      240

cgc cac agg tgg aat gtc ggc gac ctc gtc atg tgg gat aac cgc tgc      824
Arg His Arg Trp Asn Val Gly Asp Leu Val Met Trp Asp Asn Arg Cys
245                      250                      255                      260

gtg ctg cac cgc ggc agg cgc tac gat atc agc gcg cgc agg gaa ctc      872
Val Leu His Arg Gly Arg Arg Tyr Asp Ile Ser Ala Arg Arg Glu Leu
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agg cgc gcc acc acc ctc gac gac gcg gtg gtg tagtgtgctg gag      918
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<223> gene fragment

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<210> 25
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<210> 37

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<222> (1)..(44)

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<210> 46

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<223> gene fragment

<400> 46

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<210> 47

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<210> 48
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<210> 53
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WE CLAIM:

1. A DNA molecule comprising a synthetic DNA sequence,
the synthetic DNA sequence encoding an enzyme that degrades
2,4-dichlorophenoxyacetic acid to dichlorophenol, and
the synthetic DNA sequence comprising a natural microbial
sequence that encodes the enzyme in which at least a plurality of
the codons of the natural microbial sequence have been replaced
by codons more preferred by a plant.
2. The DNA molecule of Claim 1 wherein all of the codons of the
natural microbial sequence which are least preferred by the plant
have been replaced by codons more preferred by the plant.
3. The DNA molecule of Claim 1 wherein at least 50% of the codons
of the natural microbial sequence have been replaced by codons
more preferred by the plant.
4. The DNA molecule of Claim 3 wherein at least 80% of the codons
of the natural microbial sequence have been replaced by codons
more preferred by the plant.
5. The DNA molecule of any one of Claims 1-4 wherein the natural
microbial sequence is a natural bacterial sequence.
6. The DNA molecule of any one of Claims 1-4 wherein the codons
of the natural microbial sequence have been replaced by codons
more preferred by a dicotyledonous plant.
7. The DNA molecule of Claim 6 comprising the nucleotide sequence
of SEQ ID NO:2.
8. The DNA molecule of any one of Claims 1-4 wherein the codons
of the natural microbial sequence have been replaced by codons
more preferred by a monocotyledonous plant.

9. The DNA molecule of Claim 8 comprising the nucleotide sequence of SEQ ID NO:3.
10. A DNA construct comprising a synthetic DNA sequence,
the synthetic DNA sequence being operatively linked to plant gene expression control sequences,
the synthetic DNA sequence encoding an enzyme that degrades 2,4-dichlorophenoxyacetic acid to dichlorophenol, and
the synthetic DNA sequence comprising a natural microbial sequence that encodes the enzyme in which at least a plurality of the codons of the natural microbial sequence have been replaced by codons more preferred by a plant.
11. The DNA construct of Claim 10 wherein all of the codons of the natural microbial sequence which are least preferred by the plant have been replaced by codons more preferred by the plant.
12. The DNA construct of Claim 10 wherein at least 50% of the codons of the natural microbial sequence have been replaced by codons more preferred by the plant.
13. The DNA construct of Claim 12 wherein at least 80% of the codons of the natural microbial sequence have been replaced by codons more preferred by the plant.
14. The DNA construct of any one of Claims 10-13 wherein the natural microbial sequence is a natural bacterial sequence.
15. The DNA construct of any one of Claims 10-13 wherein the codons of the natural microbial sequence have been replaced by codons more preferred by a dicotyledonous plant.
16. The DNA construct of Claim 15 comprising the nucleotide sequence of SEQ ID NO:2.

17. The DNA construct of Claim 15 comprising the nucleotide sequence of SEQ ID NO:4.
18. The DNA construct of any one of Claims 10-13 wherein the codons of the natural microbial sequence have been replaced by codons more preferred by a monocotyledonous plant.
19. The DNA construct of Claim 18 comprising the nucleotide sequence of SEQ ID NO:3.
20. The DNA construct of Claim 18 comprising the nucleotide sequence of SEQ ID NO:5.
21. The DNA construct of Claim 10 which is a vector.
22. The DNA construct of Claim 10 which is a plasmid.
23. The DNA construct of Claim 10 which is pProPClSV-SAD.
24. The DNA construct of Claim 10 which is pPZP211-PNPT-311-SAD.
25. The DNA construct of Claim 10 which is pPZP211-PNPT-512-SAD.
26. The DNA construct of Claim 10 wherein the expression control sequences comprise a Peanut Chlorotic Streak Virus promoter.
27. A transgenic plant or part of a plant comprising a synthetic DNA sequence,
 - the synthetic DNA sequence being operatively linked to plant gene expression control sequences,
 - the synthetic DNA sequence encoding an enzyme that degrades 2,4-dichlorophenoxyacetic acid to dichlorophenol, and
 - the synthetic DNA sequence comprising a natural microbial sequence that encodes the enzyme in which at least a plurality of

the codons of the natural microbial sequence have been replaced by codons more preferred by a plant.

28. The plant or plant part of Claim 27 wherein the expression control sequences comprise a Peanut Chlorotic Streak Virus promoter.

29. The plant or plant part of Claim 27 wherein all of the codons of the natural microbial sequence which are least preferred by the plant have been replaced by codons more preferred by the plant.

30. The plant or plant part of Claim 27 wherein at least 50% of the codons of the natural microbial sequence have been replaced by codons more preferred by the plant.

31. The plant or plant part of Claim 30 wherein at least 80% of the codons of the natural microbial sequence have been replaced by codons more preferred by the plant.

32. The plant or plant part of any one of Claims 27-31 wherein the natural microbial sequence is a natural bacterial sequence.

33. The plant or plant part of Claim 27 which is a dicotyledonous plant or plant part and the codons of the natural microbial sequence have been replaced by codons more preferred by a dicotyledonous plant.

34. The plant or plant part of Claim 33 wherein the one or more cells comprise the nucleotide sequence of SEQ ID NO:2.

35. The plant or plant part of Claim 33 wherein the one or more cells comprise the nucleotide sequence of SEQ ID NO:4.

36. The plant or plant part of Claim 27 which is a monocotyledonous plant or plant part and the codons of the natural

microbial sequence have been replaced by codons more preferred by a monocotyledonous plant.

37. The plant or plant part of Claim 36 wherein the one or more cells comprise the nucleotide sequence of SEQ ID NO:3.

38. The plant or plant part of Claim 36 wherein the one or more cells comprise the nucleotide sequence of SEQ ID NO:5.

39. The plant or plant part of Claim 27 which is a cotton plant or plant part.

40. The plant part of Claim 27 which is a seed.

41. The plant part of Claim 27 which is a fruit.

42. A method of controlling weeds in a field containing a transgenic plant,

the method comprising applying an amount of an auxin herbicide to the field effective to control the weeds in the field,

the transgenic plant being tolerant to the auxin herbicide as a result of comprising a synthetic DNA sequence,

the synthetic DNA sequence being operatively linked to plant gene expression control sequences,

the synthetic DNA sequence encoding an enzyme that degrades 2,4-dichlorophenoxyacetic acid to dichlorophenol, and

the synthetic DNA sequence comprising a natural microbial sequence that encodes the enzyme in which at least a plurality of the codons of the natural microbial sequence have been replaced by codons more preferred by the plant.

43. The method of Claim 42 wherein the auxin herbicide is 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4-D amine.

44. The method of Claim 42 wherein the plant is a monocotyledonous plant.
45. The method of Claim 42 wherein the plant is a dicotyledonous plant.
46. The method of Claim 42 wherein the plant is a cotton plant.
47. A method of selecting transformed plant cells comprising:
providing a population of plant cells;
transforming at least some of the plant cells in the population with the DNA construct of Claim 10; and
selecting the transformed plant cells by culturing the resulting population of plant cells in a culture medium containing an auxin herbicide at a concentration selected so that transformed plant cells proliferate and untransformed plant cells do not proliferate.
48. The method of Claim 47 wherein the auxin herbicide is 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4-D amine.
49. A method of selecting transformed plants comprising:
providing a population of plants which may comprise one or more plants comprising a DNA construct according to Claim 10; and
selecting transformed plants by applying an amount of an auxin herbicide to the population of plants, the amount of herbicide being selected so that transformed plants grow and growth of untransformed plants is inhibited.
50. The method of Claim 49 wherein the auxin herbicide is 2,4-dichlorophenoxyacetic acid (2,4-D) or 2,4-D amine.

1/2

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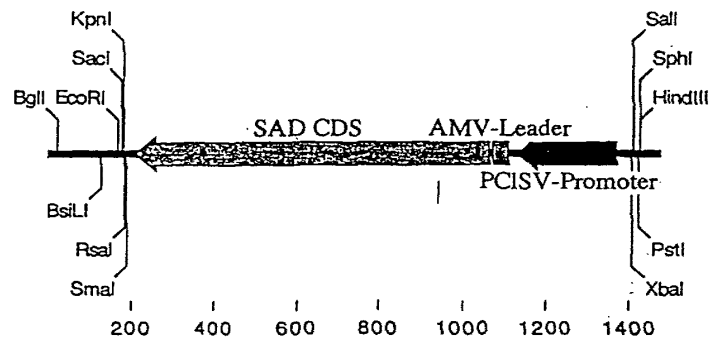


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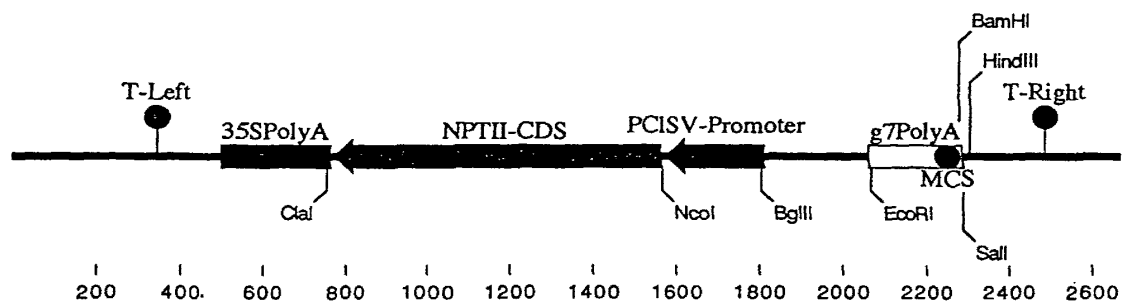


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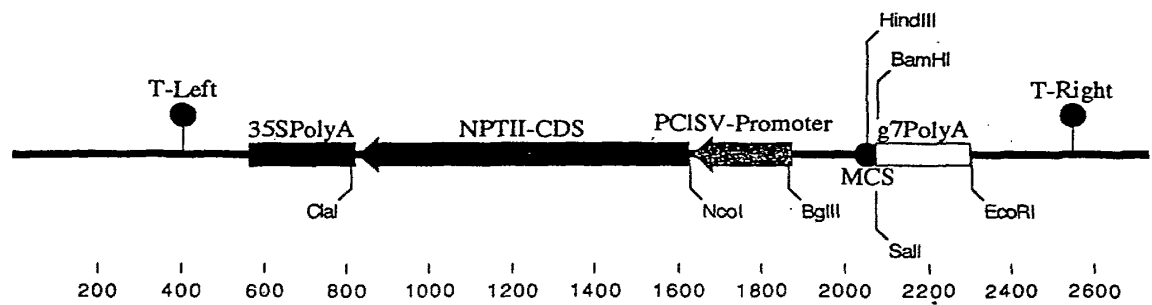


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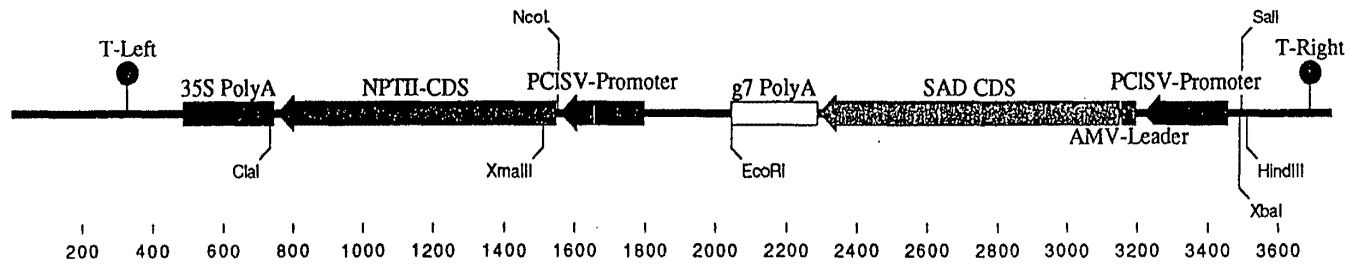
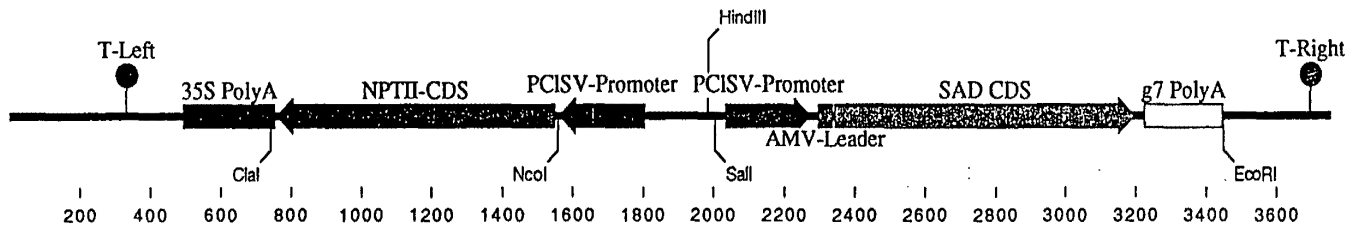


Fig 5





All Databases

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EMBL Nucleotide Sequence Database

EBI EMBL Nucleotide Sequence Database Sequence Version Archive

The EMBL Sequence Version Archive is a repository of all entries which have ever appeared in the EMBL Nucleotide Sequence Database. You can use this page to browse the archive or use the [batch retrieval form](#).

F.A.Q.

Accession Number or Sequence Version: ☐ case sensitiveSnapshot at day-month-year (e.g. 30-11-1998 or 30-NOV-1998) [Previous <<](#)

AJ628859

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This flat file was issued: 03-MAR-2004 Rel: 79

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Sphingomonas herbicidovorans MH: a versatile phenoxyalkanoic acid herbicide degrader

HPE Kohler

EAWAG, Swiss Federal Institute for Environmental Sciences and Technology, Ueberlandstrasse 133, CH-8600 Dübendorf, Switzerland

Sphingomonas herbicidovorans MH was isolated from a dichlorprop-degrading soil column. It is able to grow on phenoxyalkanoic acid herbicides, such as mecoprop, dichlorprop, 2,4-D, MCPA, and 2,4-DB. Strain MH utilizes both enantiomers of the chiral herbicides mecoprop and dichlorprop as sole carbon and energy sources. Enantiomer-specific uptake systems are responsible for transporting the acidic substrates across the cell membrane. Catabolism is initiated by two enantiomer-specific α -ketoglutarate-dependent dioxygenases that catalyze the cleavage of the ether bond of the respective enantiomer to yield the corresponding phenol and pyruvate. Therefore selective degradation of the enantiomers of mecoprop and dichlorprop by strain MH is not only due to enantioselective catabolism but also to enantioselective transport.

Keywords: *Sphingomonas herbicidovorans* MH; phenoxyalkanoic acid herbicides; enantioselectivity; transport; α -ketoglutarate-dependent dioxygenase

Introduction

Sphingomonas herbicidovorans MH has been recently isolated and described [12,28]. Our studies of strain MH, which I summarize in this short review, are mainly driven by two reasons. First, strain MH degrades phenoxyalkanoic acid herbicides such as mecoprop, dichlorprop, 2,4-D, MCPA, and 2,4-DB (Figure 1, Table 1) [12,27,28]. The broad catabolic capabilities of strain MH with respect to the degradation of these herbicides, which are often found in leachates of landfills [5,10,13,29] and as groundwater contaminants [7], make this strain a potential candidate for bioremediation. Second, strain MH metabolizes the chiral phenoxypropanoic acid herbicides mecoprop and dichlorprop in an enantioselective manner. The effect of chirality on the metabolism of chiral pollutants, the importance of treating enantiomers separately, and the necessity of considering stereochemistry when studying degradation and environmental fate of chiral compounds have been emphasized in recent reviews [2,3,14,16]. Strain MH is an ideal system for studying the effect of chirality on the bacterial metabolism of chiral herbicides.

The chlorine-substituted phenoxyacetic acids 2,4-D, 2,4,5-T, and MCPA (Figure 1) were introduced as selective weed killers in the 1940s [18,26]. They are active against many broad-leaved weeds and are commonly used for the control of weeds in cereal crops, in grass pastures, and in lawns. The chiral 2-phenoxypropanoic acids mecoprop, dichlorprop, and fenoprop (Figure 1) were introduced in the 1950s for controlling certain weed species that were unaffected by the phenoxyacetic acid herbicides [18]. The herbicidal action of the phenoxyalkanoic acid herbicides is based on their ability to mimic natural plant growth regulators or

auxins [18]. These synthetic auxins induce several abnormalities in growth and plant structure; they cause dedifferentiation and initiation of cell division in certain mature cells and inhibit cell division in primary meristems, processes that ultimately lead to cell death. As early as 1953, studies on the stereospecificity of plant growth regulators revealed that the herbicidal activity of mecoprop and dichlorprop is associated only with the *R* enantiomer [19]. The phenoxyalkanoic acid herbicides are among the most widely used herbicides to control weeds in cereal crops throughout the world [26]. In the European Union (EU), the estimated total usage of 2,4-D, MCPA, mecoprop, and dichlorprop exceeded 11 000 tons in 1991 [7].

Strain MH belongs to the genus *Sphingomonas* [23–25], which appears to be ubiquitous in soil, water, and sediments. *Sphingomonas* strains isolated from these environments have broad catabolic capabilities that make them potentially useful for bioremediation and waste treatment [4]. The unique ability of *S. herbicidovorans* MH to cleave the ether bond of various phenoxyalkanoic acid herbicides makes it an interesting and fitting addition to this genus.

Taxonomic characteristics of strain MH

Sphingomonas herbicidovorans MH was isolated by Horvath *et al* [12]. They repeatedly cultivated a sample from a dichlorprop-degrading soil column (nine transfers) and used the sample to inoculate a chemostat. After 3 weeks of incubation in continuous culture, they isolated a dichlorprop-degrading strain from the effluent of the chemostat. Strain MH consists of Gram-negative, catalase- and oxidase-positive, yellow-pigmented, non-motile, and non-spore-forming rods [12]. It does not ferment carbohydrates, does not accumulate poly- β -hydroxybutyric acid under nitrogen-deficient conditions, does not use nitrate as an electron acceptor, and does not utilize complex carbohydrates [12]. Horvath *et al* [12] also noticed that strain

Correspondence: HPE Kohler, Department of Microbiology, EAWAG, Überlandstrasse 133, CH-8600 Dübendorf, Switzerland
Received 7 May 1999; accepted 11 August 1999

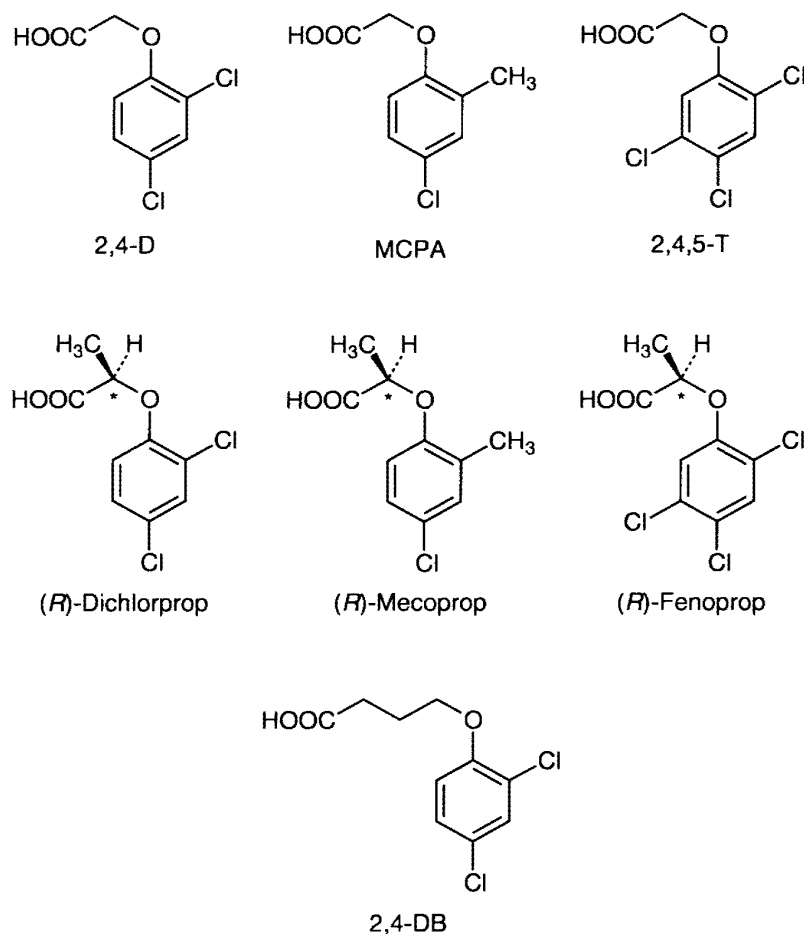


Figure 1 Structural formulas of the achiral phenoxyacetic acid herbicides 2,4-dichlorophenoxyacetic acid (2,4-D), 4-chloro-2-methylphenoxyacetic acid (MCPA), 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), of the achiral phenoxybutanoic acid herbicide 4-(2,4-dichlorophenoxy)butanoic acid (2,4-DB), and of the chiral phenoxypropanoic acid herbicides (R)-2-(2,4-dichlorophenoxy)propanoic acid [(R)-dichloroprop], (R)-2-(4-chloro-2-methylphenoxy)propanoic acid [(R)-mecoprop], and (R)-2-(2,4,5-trichlorophenoxy)propanoic acid [(R)-fenoprop]. Asterisks mark the stereogenic centers.

MH releases a brownish water-soluble pigment into complex nutrient media. They tentatively identified strain MH as a *Flavobacterium* sp. Additional work on the taxonomic characteristics of strain MH by Zipper *et al* [28] and the Deutsche Stammsammlung von Mikroorganismen und Zellkulturen (DSMZ) [28] revealed that in addition to the characteristics mentioned above, strain MH is aminopeptidase-positive and alcohol dehydrogenase- and urease-negative, lyses only esculin but not gelatin or DNA, is not able to grow anaerobically, and does not form acids from sugars. Analysis of the cellular fatty acids showed that strain MH belongs to the *Sphingomonas* group within the α -subgroup of the proteobacteria [28]. The partial sequence of the gene for the 16S rRNA has the highest degree of similarity to the one for the 16S rRNA of *Rhizomonas suberifaciens* and *S. yanoikuyae*. DNA-DNA hybridization experiments between strain MH and the type strains of these two species showed that strain MH belongs to a new species within the group [28]. Although the highest identity was observed with *R. suberifaciens* (37.4%), the strain was named *S. herbicidovorans* because the genus name *Rhizomonas* is a

nomen rejiciendum (opinion 14) and, therefore, usage of this name is in violation of rule 28a of the *International Code of Nomenclature of Bacteria* [17]. The name *Sphingomonas herbicidovorans* MH was validated by inclusion on validation list No. 61 of the *International Journal of Systematic Bacteriology* [1].

Growth characteristics

Growth at 30°C on nutrient agar plates produces circular, bright-yellow colonies after 3 days; when the plates are incubated longer, the colonies change to a flower-like shape. Cells secrete a brownish pigment when growing on solid nutrient medium. In mineral salts medium with mecoprop as the sole carbon and energy source, strain MH requires supplements of vitamins and peptone for reproducible growth [28].

Table 1 lists growth substrates of *S. herbicidovorans* MH. Where available, growth rates, yields, and substrate consumption rates are included in the table. Strain MH is able to grow on the most important phenoxyalkanoic acid

Table 1 Growth characteristic of *S. herbicidovorans* MH

Growth substrate	Growth rate (h ⁻¹)	Substrate consumption rate (μM h ⁻¹)	Yield (g dry weight g ⁻¹ substrate)	Reference
(<i>R</i>)-Dichlorprop	0.082 ± 0.006	28	0.37 ± 0.06	[27]
(<i>S</i>)-Dichlorprop	0.036 ± 0.001	12	0.35 ± 0.02	[27]
Racemic mixture of dichlorprop ^a	NA ^b	NA	0.29 ± 0.03	[27]
-(<i>R</i>)-Dichlorprop ^a	0.043 ± 0.003	22	NA	[27]
-(<i>S</i>)-Dichlorprop ^a	0.029 ± 0.004	13	NA	[27]
Racemic mixture of dichlorprop	0.2 ^c	35	ND ^d	[12]
(<i>R</i>)-Mecoprop	0.032	14	0.30 ± 0.15	[28]
(<i>S</i>)-Mecoprop	0.039	13	0.35 ± 0.05	[28]
Racemic mixture of mecoprop ^e	0.016 ^f	NA	0.30 ± 0.04	[28]
-(<i>R</i>)-Mecoprop ^g	NA	6	NA	[28]
-(<i>S</i>)-Mecoprop ^g	NA	5	NA	[28]
2,4-D	0.133 ± 0.003	54	0.19 ± 0.05	[27]
MCPA	+ ^h	ND	ND	[28]
2,4-DB	+	ND	ND	[12]
2,4,5-T	- ^h	NA	NA	[12]

^aDiauxic growth; a growth rate was calculated for each enantiomer.^bNA, not applicable.^cThis growth rate is probably not correct. Recalculation of the data given in the reference showed that the growth rate must be between 0.065 and 0.115 per h.^dND, not determined.^eNo indication of diauxic growth; one growth rate was calculated.^fIn this case the growth rate was affected by the concentration, which for each enantiomer was only half the value of what it was in the experiments with pure mecoprop enantiomers. Additional experiments showed increased growth rates in experiments with higher (*RS*)-mecoprop concentrations [28].^g+, Strain MH grew on the compound.^h-, Strain MH did not grow on the compound.

herbicides, such as mecoprop, dichlorprop, 2,4-D, MCPA, and 2,4-DB (Table 1, Figure 1).

S. herbicidovorans MH utilizes both enantiomers of mecoprop and dichlorprop as sole carbon and energy sources for growth [12,27,28]. However, growth experiments inoculated with cells that were grown on complex media and with the single pure enantiomers or the racemic mixtures as substrates revealed enantioselectivity; strain MH always degrades the *S* enantiomers before the *R* enantiomers. Growth on (*S*)-mecoprop is complete after about 3.5 days whether the *R* enantiomer is present or not. With racemic mecoprop as the growth substrate, degradation of the enantiomers is sequential, with (*S*)-mecoprop being degraded first [28]. However, the growth curve does not show indications of diauxic growth and, therefore, only one growth rate is reported in Table 1 for racemic mecoprop. (*R*)-Mecoprop is only slowly degraded during the first 50 h of such experiments, but its degradation rate markedly increases after the complete consumption of (*S*)-mecoprop. With pure (*R*)-mecoprop as the sole carbon and energy source, cells start to grow after a lag phase of about 7 days. Once growth commences, the cells grow as fast with (*S*)-mecoprop as the growth substrate (Table 1) [28]. Basically the same results were obtained with dichlorprop as the sole carbon and energy source [27]. As the growth substrate, (*S*)-dichlorprop completely disappears from the culture

medium after 43 h of incubation, while in experiments with (*R*)-dichlorprop as the growth substrate, a lag period of about 60 h is evident. When strain MH is offered racemic dichlorprop as the growth substrate, it degrades both enantiomers; the *S* enantiomer is preferentially degraded but at a slower rate (Table 1) [27]. In contrast to growth on racemic mecoprop, growth on racemic dichlorprop is diauxic and, therefore, two growth rates are reported in Table 1. Generally, chloride release is stoichiometric and strain MH does not excrete any detectable metabolites [12,27,28]. The levels of dissolved organic carbon at the beginning and at the end of a growth experiment usually correspond well to a complete conversion of dichlorprop and mecoprop [28].

Additional growth experiments showed that strain MH grows best on 2,4-D [27] (Table 1). Typically, growth on 2,4-D starts without a lag period and is complete after about 15 h of incubation.

Enantioselective metabolism of mecoprop and dichlorprop

Biochemical investigations focused on enzyme activities that turn over the mecoprop enantiomers [20]. *S. herbicidovorans* MH was grown on pure enantiomers and the harvested cells were disrupted. The crude cell extracts were tested for the existence of enzyme activities that were able to convert either (*R*)- or (*S*)-mecoprop. Cell extracts of *Sphingomonas herbicidovorans* MH grown on (*R*)-mecoprop contain an enzyme activity that selectively converts (*R*)-mecoprop to 4-chloro-2-methylphenol, whereas the enzyme activity in cell extracts of (*S*)-mecoprop-grown cells is selective for the *S* enantiomer. Both reactions depend on α -ketoglutarate and ferrous ions. Besides 4-chloro-2-methylphenol, pyruvate and succinate are formed as products of the reactions [20]. Labeling experiments with ¹⁸O₂ revealed that both enzyme activities catalyze a dioxygenation reaction. The appearance of ¹⁸O label in pyruvate and not in phenol suggests that the oxidative cleavage of the ether-bond proceeds through hydroxylation at the C-2 carbon of the propanoic acid side chain. The resulting unstable intermediate should then easily decompose to 4-chloro-2-methylphenol and pyruvate [20]. This suggestion was also in agreement with a general reaction mechanism for α -ketoglutarate-dependent dioxygenases. The reaction is thought to start with the formation of a ferryl oxidant (Fe_{IV}=O) by the binding of oxygen to the iron cofactor, a process that is linked to oxidative decarboxylation of α -ketoglutarate to succinate [6,11,21,22]. Therefore, Nickel *et al* [20] proposed a degradation scheme based on two distinct α -ketoglutarate-dependent dioxygenases for the initial transformation of (*R*)- and (*S*)-mecoprop to 4-chloro-2-methylphenol and pyruvate by *S. herbicidovorans* MH (Figure 2) [20]. Both enzyme activities seem to share the overall chemistry with 2,4-D dioxygenase (TfdA), the enzyme catalyzing the first step of the degradation of 2,4-D by *R. eutropha* JMP134 [8,9], but not the substrate specificity. Whereas TfdA preferentially transforms 2,4-D to 2,4-dichlorophenol and glyoxylate (Figure 2) [8,9], the enzyme activities present in cell extracts of *S. herbicidovorans* MH specifically turn over either the *R* or the *S* enantiomer of mecoprop and show

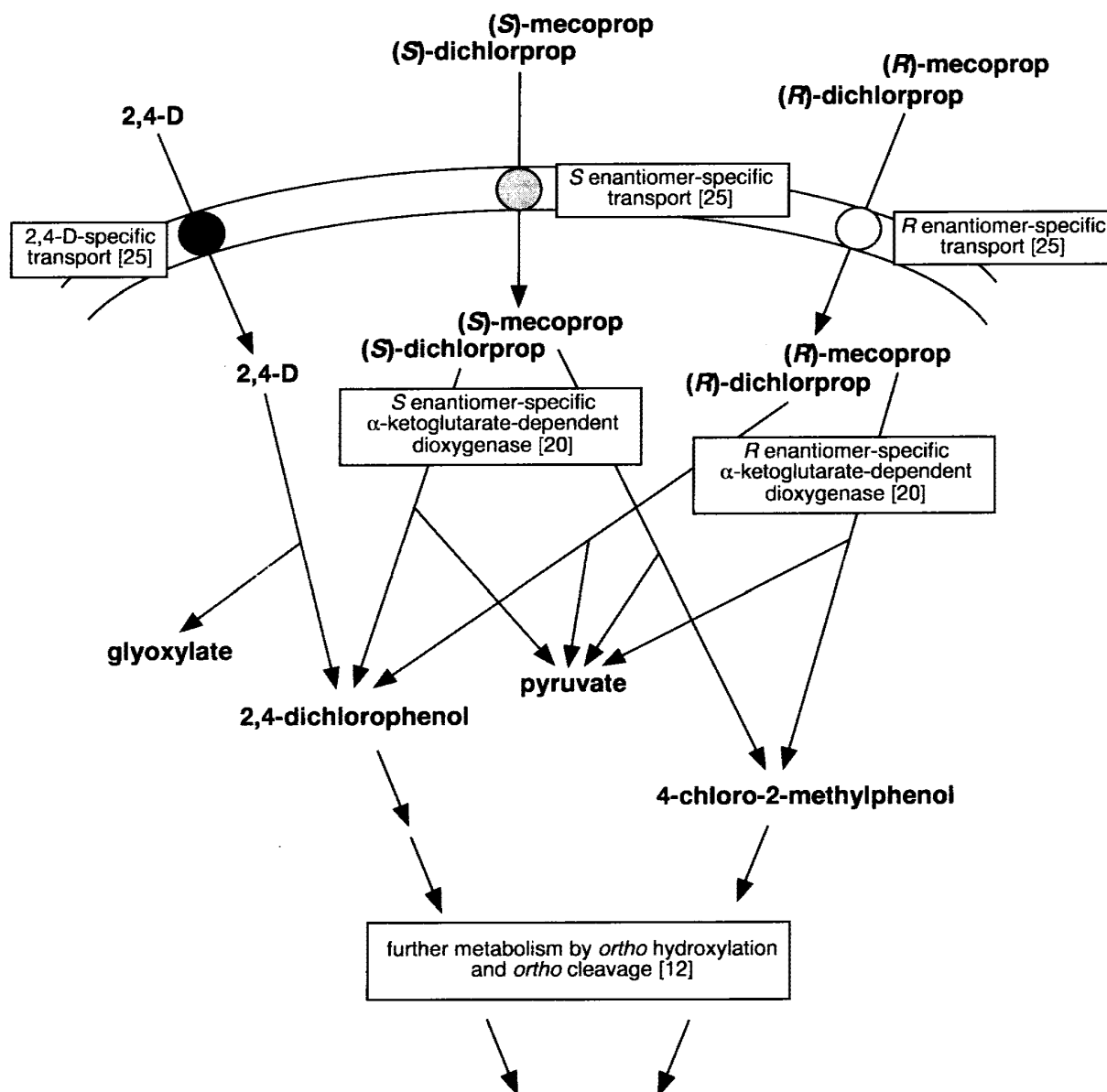


Figure 2 Model proposed for the uptake and metabolism of (*R*)- and (*S*)-dichlorprop, (*R*)- and (*S*)-mecoprop, and 2,4-D by *S. herbicidovorans* MH. Strain MH has three inducible proton gradient-driven uptake systems; one for (*R*)-dichlorprop and (*R*)-mecoprop, one for (*S*)-dichlorprop and (*S*)-mecoprop, and a third one for 2,4-D [27]. Once the compounds enter the cell, specific α-ketoglutarate-dependent dioxygenases enantioselectively catalyze cleavage of the ether bonds [20].

less activity with 2,4-D as the substrate [20]. (*R*)- and (*S*)-dichlorprop are also substrates of the (*R*)-enantiomer-specific enzyme and the (*S*)-enantiomer-specific enzyme, respectively; in these cases, 2,4-dichlorophenol and pyruvate were the common products (Figure 2) [15,20].

Enantioselective uptake of mecoprop and dichlorprop

Transport studies with ¹⁴C-labeled dichlorprop enantiomers and with ¹⁴C-labeled 2,4-D revealed that the uptake of (*R*)-dichlorprop, (*S*)-dichlorprop, and 2,4-D by *S. herbi-*

cidovorans MH is inducible [27]. The results from these transport studies clearly demonstrated that the uptake of dichlorprop is enantioselective and that there must be a separate uptake system for each enantiomer [27]. Additionally, Zipper *et al* [27] showed that the rates of uptake of 2,4-D by 2,4-D-grown cells in response to increasing 2,4-D concentrations are in accordance with saturation kinetics. The same is true for the transport of (*R*)-dichlorprop and the transport of (*S*)-dichlorprop into (*R*)-dichlorprop-grown and (*S*)-dichlorprop-grown cells, respectively. Experiments on the substrate specificity of the putative transport systems revealed that (*R*)-dichlorprop uptake is inhibited by (*R*)-

mecoprop but not by (*S*)-mecoprop, (*S*)-dichlorprop, or 2,4-D. On the other hand, (*S*)-dichlorprop transport is inhibited by (*S*)-mecoprop but not by (*R*)-dichlorprop, (*R*)-mecoprop, or 2,4-D [27]. From these results, Zipper *et al* [27] concluded that the (*R*)-dichlorprop carrier also transports (*R*)-mecoprop and that the (*S*)-dichlorprop carrier also transports (*S*)-mecoprop. Apparently, the transport systems are specific with respect to the substitution and the configuration at the stereogenic center (C-2 atom of the propanoic acid side chain) and rather unspecific with respect to the aromatic moiety of the phenoxypopropanoic acids [27]. Moreover, transport of (*R*)-dichlorprop, (*S*)-dichlorprop, and 2,4-D is completely inhibited by various uncouplers and by the ionophore nigericin but is only marginally inhibited by the ionophore valinomycin and the ATPase inhibitor *N,N*-dicyclohexylcarbodiimide [27]; these results are strong indications for the involvement of active transport. Zipper *et al* [27] concluded and summarized that the first step in the degradation of dichlorprop, mecoprop, and 2,4-D in *S. herbicidovorans* MH is active transport and that three inducible, proton-gradient driven uptake systems exist; one for (*R*)-dichlorprop and (*R*)-mecoprop, another for (*S*)-dichlorprop and (*S*)-mecoprop, and a third for 2,4-D (Figure 2).

Concluding remarks

The reaction sequence illustrated in Figure 2 demonstrates that selective degradation of the enantiomers of mecoprop and dichlorprop by the soil isolate *S. herbicidovorans* MH [28] is not only due to enantioselective catabolism [20] but also to enantioselective transport [27].

Taking into consideration the fact that the toxicity and the effect on biological targets might strongly differ for the enantiomers of a chiral pesticides or drug [16], study of enantioselective metabolism on the molecular level well deserves further emphasis.

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      cgctacctga cgcgcaccac ggtcgggtggc gtgcgcccgg cgcgctag      888

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Localization and Characterization of Two Novel Genes Encoding Stereospecific Dioxygenases Catalyzing 2(2,4-Dichlorophenoxy)propionate Cleavage in *Delftia acidovorans* MC1

Kathleen M. Schleinitz,¹ Sabine Kleinsteuber,^{1*} Tatiana Vallaeys,^{2,3} and Wolfgang Babel¹

Department of Environmental Microbiology, UFZ Centre for Environmental Research Leipzig-Halle, Leipzig, Germany,¹ and Institut National de la Recherche Agronomique² and Laboratoire de Génomique des Microorganismes Pathogène, Institut Pasteur,³ Paris, France

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Two novel genes, *rdpA* and *sdpA*, encoding the enantiospecific α -ketoglutarate dependent dioxygenases catalyzing *R,S*-dichlorprop cleavage in *Delftia acidovorans* MC1 were identified. Significant similarities to other known genes were not detected, but their deduced amino acid sequences were similar to those of other α -ketoglutarate dioxygenases. RdpA showed 35% identity with TauD of *Pseudomonas aeruginosa*, and SdpA showed 37% identity with TfdA of *Ralstonia eutropha* JMP134. The functionally important amino acid sequence motif HX(D/E)₂₃₋₂₆(T/S)₁₁₄₋₁₈₃HX₁₀₋₁₃R/K, which is highly conserved in group II α -ketoglutarate-dependent dioxygenases, was present in both dichlorprop-cleaving enzymes. Transposon mutagenesis of *rdpA* inactivated *R*-dichlorprop cleavage, indicating that it was a single-copy gene. Both *rdpA* and *sdpA* were located on the plasmid pMC1 that also carries the lower pathway genes. Sequencing of a 25.8-kb fragment showed that the dioxygenase genes were separated by a 13.6-kb region mainly comprising a Tn501-like transposon. Furthermore, two copies of a sequence similar to IS91-like elements were identified. Hybridization studies comparing the wild-type plasmid and that of the mutant unable to cleave dichlorprop showed that *rdpA* and *sdpA* were deleted, whereas the lower pathway genes were unaffected, and that deletion may be caused by genetic rearrangements of the IS91-like elements. Two other dichlorprop-degrading bacterial strains, *Rhodospirillum rubrum* strain P230 and *Sphingobium herbicidovorans* MH, were shown to carry *rdpA* genes of high similarity to *rdpA* from strain MC1, but *sdpA* was not detected. This suggested that *rdpA* gene products are involved in the degradation of *R*-dichlorprop in these strains.

Chlorinated phenoxyalkanoic acids such as 2,4-dichlorophenoxyacetate (2,4-D), 2-methyl-4-chlorophenoxyacetate (MCPA), 2(2,4-dichlorophenoxy)propionate (dichlorprop), and 2(2-methyl-4-chlorophenoxy)propionate (mecoprop) have been widely used as broad leaf herbicides since their introduction in the 1940s. The ability to degrade the phenoxyacetate herbicides is widespread among bacteria (16). In contrast, breakdown of the structurally very similar chiral phenoxypropionate derivatives is far less widespread, and only few strains able to mineralize these compounds have been described (9, 21, 30, 35, 42, 50).

Degradation of phenoxyalkanoate herbicides is initiated by the etherolytic cleavage of the side chain yielding the respective phenol (Fig. 1). Subsequently, conversion into intermediates of the tricarboxylic acid cycle takes place via the modified *ortho*-pathway (16). Side chain cleaving specificity is crucial and determines the range of substrates that can be mineralized by a given microorganism.

Thus far, two types of 2,4-D-cleaving enzymes have been described. In *Ralstonia eutropha* JMP134, 2,4-D cleavage is catalyzed by a well-studied Fe(II) and α -ketoglutarate (α KG)-dependent dioxygenase, which is encoded by *tfdA* (13, 20, 39, 45, 59). The presence of the functionally essential motif HX(D/

E)₂₃₋₂₆(T/S)₁₁₄₋₁₈₃HX₁₀₋₁₃R/K indicates that TfdA is related to several other (referred to as group II) α KG-dependent dioxygenases (18). Recently, a three-dimensional model of the active site was proposed (10) on the basis of predictions made from site-directed mutagenesis studies (20) and secondary structure predictions (10). The identity of the suggested substrate-binding residues was confirmed by site-directed mutagenesis and inactivation studies (8). The *tfdA* genes are widespread among bacteria (17, 31, 33, 36, 39, 42, 47, 53, 54, 55, 56), also among strains unable to degrade 2,4-D (19). Recently, several α -proteobacterial strains were shown to carry *tfdA* α genes, which are *tfdA*-like, showing between 46 and 60% nucleotide identity to the canonical *tfdA* genes and forming a phylogenetic clade distinct from *tfdA* (22, 23). Three other genes, clustered as *cadABC*, were found to encode a second type of 2,4-D-cleaving enzyme, a putative monooxygenase responsible for 2,4-D cleavage in *Bradyrhizobium* sp. strain HW13 (24).

Studies on *Sphingobium* (formerly *Sphingomonas* [48]) *herbicidovorans* MH and *Delftia acidovorans* MC1 indicated that enantiospecific dichlorprop and mecoprop cleavage is catalyzed by Fe(II)- and α KG-dependent dioxygenases (35, 38). The enzymes have been biochemically characterized (34, 38, 57, 58), but no data regarding the genetic background are yet available. Thus far, the dioxygenases have been assumed to be *tfdA* gene products (39), because some dichlorprop- and mecoprop-degrading strains carry *tfdA* genes (36, 39) and cell extracts of *R. eutropha* strain JMP134 showed some specificity

* Corresponding author. Mailing address: UFZ Centre for Environmental Research Leipzig-Halle, Department of Environmental Microbiology, Permoserstr. 15, 04318 Leipzig, Germany. Phone: 49-341-235-2191. Fax: 49-341-235-2247. E-mail: sabine.kleinsteuber@ufz.de.

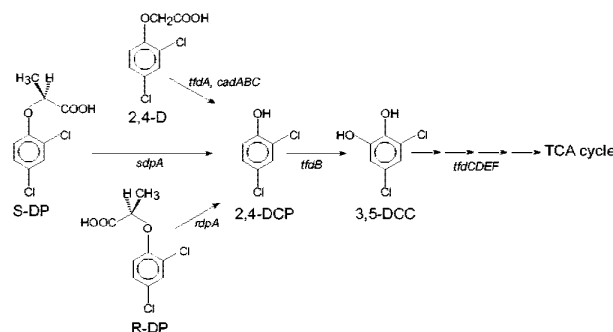


FIG. 1. Modified *ortho*-pathway for the degradation of chlorinated phenoxyalkanoate herbicides. 2,4-D, 2,4-dichlorophenoxyacetate; R-DP and S-DP, R- and S-enantiomers of 2(2,4-dichlorophenoxy)propionate, respectively; 2,4-DCP, 2,4-dichlorophenol; 3,5-DCC, 3,5-dichlorocatechol; TCA, tricarboxylate.

toward cleaving *S*-dichlorprop (39). However, no activity for *S*-mecoprop or the *R*-enantiomers was detected in strain JMP134, and the organism was unable to grow on phenoxypropionate herbicides. Recently, available N-terminal and internal amino acid sequences of the enantiospecific dioxygenases from *D. acidovorans* MC1 showed only low similarities to TfdA enzymes (57), and *tfdA* genes were not detected in this strain (36). This suggested that genes other than *tfdA* are involved in dichlorprop degradation in strain MC1.

In *D. acidovorans* MC1 the first step in dichlorprop degradation was found to be highly unstable and easily lost under nonselective conditions (36). Loss of dichlorprop-cleaving activity was correlated with a deletion in the plasmid pMC1, and it was therefore hypothesized that the deleted fragment contains the genes encoding dichlorprop cleavage (36).

Based on the recently available peptide sequences of the *R*- and *S*-dichlorprop-cleaving dioxygenases, we investigated the genetic background of dichlorprop cleavage in *D. acidovorans* MC1. Two novel genes encoding the *R*- and *S*-dichlorprop-cleaving dioxygenases in *D. acidovorans* MC1 were identified and localized. The phylogenetic relationship of the *R*,*S*-dichlorprop-cleaving dioxygenases to other α -ketoglutarate-dependent dioxygenases was analyzed. The organization of these genes within the genome of strain MC1, as well as in a mutant deficient in dichlorprop cleavage, was investigated with regard to implications for the instability of this trait.

MATERIALS AND METHODS

Strains, plasmids, and culture conditions. *D. acidovorans* MC1 and *Rhodospirillum rubrum* sp. strain P230, isolated from contaminated building rubble (9, 35), *D. acidovorans* MC1-DP⁻ (a mutant of strain MC1 arisen by spontaneous loss of dichlorprop-cleaving ability), and *S. herbicidovorans* MH, isolated from soil (21), were all grown on MM agar (36) containing 0.5 mM dichlorprop at 30°C. *Escherichia coli* TOP10F⁺ (pCR2.1; Invitrogen) was used for cloning PCR products. The cosmid library was constructed in *E. coli* XL1-Blue MR (Stratagene). *E. coli* XL1-Blue MRF⁺ (Stratagene) was used for maintaining pScosPA1. The vector pScosPA1 is a new broad-host-range cosmid identical to pSBC1 (43) except for a gentamicin resistance instead of the tetracycline resistance (*E. Mahenthalingam*, unpublished data). A shotgun library of a cosmid was constructed by using the vector pCDNA2.1 (Invitrogen). *E. coli* S17-1 (pAG408::gfp [46]) was used in transposon mutagenesis experiments. *E. coli* strains were cultivated on Luria-Bertani (LB) agar at 37°C with the addition of appropriate antibiotics. Overnight cultures were prepared in liquid LB medium containing appropriate antibiotics at 37°C (*E. coli* strains) or 30°C (dichlorprop-degrading

strains) on a rotary shaker. Antibiotics and dichlorprop were filter sterilized through a 0.2- μ m-pore-size Minisart cellulose acetate filter (Sartorius) and added at a concentration of 100 μ g/ml.

Mercury resistance was examined on LB agar plates containing 0.1 mM HgCl₂ incubated at 30°C for 48 h. *R. eutropha* JMP134(pJP4) and *R. eutropha* JM222 were used as positive and negative controls, respectively.

Construction of gene libraries. A genomic library of *D. acidovorans* MC1 was constructed by using cosmid pScosPA1, as described elsewhere (43). The average insert size of library clones was 34 kb. A total of 2,592 clones were picked into 96-well plates. After overnight growth 8% (vol/vol) dimethyl formamide was added, and the cosmid library was stored at -80°C.

A shotgun library of cosmid pScos-R5 was constructed in pCDNA2.1 according to the method of Buchrieser et al. (4) by using cosmid DNA isolated as described elsewhere (2, 3).

Transposon mutagenesis. The transposon of the suicide vector pAG408, a miniTn5 derivative, carries a promoterless *gfp* gene (46) and was used for transposon mutagenesis of strain MC1. Mutagenesis was performed according to standard procedures (40). Overnight cultures of the donor and the recipient grown in LB medium supplemented with antibiotics (donor) were centrifuged, washed, and resuspended in LB medium to a final optical density at 600 nm (OD₆₀₀) of 1.0. Equal volumes of the donor and the recipient were mixed, and 100 μ l of the mixture was plated out on LB agar, followed by incubation at 30°C for 6 h. The bacterial lawn was then washed from the plates with MM medium. Dilution series were prepared in MM and 100 μ l of the 10⁻³ to the 10⁻⁵ dilutions was plated on selective MM agar plates containing 100 μ g of gentamicin/ml, 27.8 mM fructose, and 0.5 mM dichlorprop. After incubation at 30°C for 6 days green fluorescing mutants were isolated and tested for dichlorprop degradation by high-pressure liquid chromatography as described elsewhere (60). In mutant clones able to degrade one of the enantiomers but not the other the region flanking the transposon was PCR amplified and sequenced.

DNA amplification. Degenerate primers (Table 1) were designed from N-terminal and internal peptide sequences of the enantiospecific dioxygenases of *D. acidovorans* MC1 (57) for PCR amplification of *rdpA* and *sdpA*. According to preliminary alignments of these peptide sequences with TfdA and TauD, the expected PCR fragment sizes were ca. 350 bp (*R*-dichlorprop dioxygenase gene) and 540 bp (*S*-dichlorprop dioxygenase gene). All primers were synthesized by MWG Biotech. Total genomic DNA used as a template was prepared from colonies grown on LB agar as described elsewhere (25). PCR was performed by using the *Taq* PCR Mastermix kit (Qiagen) and a PTC-200 thermal cycler (MJ Research). First, 100 pmol of each degenerate primer was added to 25 μ l of PCR sample. The temperature gradient program involved an initial denaturation step at 94°C for 3 min, followed by 36 cycles of 94°C for 45 s, 50 to 70°C for 45 s, and 72°C for 2 min, with a final elongation step of 72°C for 6 min. Amplification of an *rdpA* gene fragment with the primers rdp.fg and rdp.r involved an initial denaturation step at 94°C for 3 min, followed by 36 cycles of 94°C for 45 s, 54°C for 45 s, and 72°C for 1 min, with a final elongation step of 72°C for 6 min. Amplification of an *sdpA* gene fragment with the primers sdp.fg and sdp.rA was the same as for *rdpA* amplification, except that the annealing step was performed at 60°C and that elongation lasted 90 s.

The *rdpA* genes from *S. herbicidovorans* MH and *Rhodospirillum rubrum* sp. strain P230 were amplified with primers rdp.fgw (5'-atg gta ggt ctc aaA TGC ATG CTG CAC TGT CCC CC-3'; lowercase letters denote a linker sequence) and rdp.rev (5'-atg gta ggt ctc agc gct CTA GCG CGC CGG GCG CAC G-3'). Amplification was the same as for the *rdpA* fragment except for the annealing step being performed at 60°C for 30 s.

Regions flanking Tn5 transposon insertion sites were amplified by ST-PCR (6). To each sample either Tn5.outF (5'-GGC AGA TCT GAT CAA GAG-3' [27]) annealing to the I end of the transposon or Tn5.outR (5'-CCG CAC TTG TGT ATA AGA GTC-3') designed from the O end of the transposon were added as specific primers. Either of the following FP primers (44) was added as a random primer: FP-1, 5'-AGT TCA AGC TTG TCC AGG AAT TC(N), GGC CT-3'; FP-2, 5'-AGT TCA AGC TTG TCC AGG AAT TC(N), GCG CT-3'; FP-3, 5'-AGT TCA AGC TTG TCC AGG AAT TC(N), CCG GT-3'; and FP-4, 5'-AGT TCA AGC TTG TCC AGG AAT TC(N), CGC GT-3'.

PCR products for the use as hybridization probes were generated as follows: the *mer* operon was partially amplified with primers mer.f (5'-CAC CAG TTC AGA CAG CAC G-3') and mer.r (5'-ACC GGT GAA ACC AAG TCC-3') amplifying a 1,450-bp fragment. The IS91-like element was partially amplified by using the primers IS.f (5'-ATG TCA GCC ACT TCC AAG C-3') and IS.r (5'-GGA GGC AGT ATG CAT TTG G-3') amplifying a 1,622-bp fragment. The PCR program for both fragments involved an initial denaturation step of 94°C for 3 min, followed by 36 cycles of 94°C for 45 s, 60°C for 45 s, and 72°C for 2 min, with a final elongation step of 72°C for 6 min. Amplification of *tfdB*, *tfdC*, and

TABLE 1. Degenerate primers designed for the amplification of *rdpA* and *sdpA*

Peptide or primer	Sequence ^a	Position ^b
RDP internal sequence 2 rdp_r	W E T L S P T M Q A T I E G L N 3'-AC GTY CGN TGN TAI CTY CC-5'	162-177
RDP internal sequence 3 rdp_fa ^c rdp_fg ^c	N E I L D A F H T Y Q V I Y F P 5'-GCI TTY CAY ACN TAY CAA GT-3' 5'-GCI TTY CAY ACN TAY CAG GT-3'	43-58
SDP N terminus sdp_fC ^c sdp_fT ^c	M Q T T L Q I T P T G A T L G A 5'-ATG CAR ACI ACI CTG CAR ATC AC-3' 5'-ATG CAR ACI ACI CTG CAR ATT AC-3'	1-16
SDP internal sequence 1 sdp_rA ^c sdp_rG ^c	L G H V Q Q A G S A Y I 3'-GTA CAN GTY GTY CGN CC-5' 3'-GTA CAN GTY GTY CGN CC-5'	193-204

^a As described previously (57). IUPAC ambiguity codes: R = A or G, Y = C or T, N = any base, I = inosine.

^b Alignment is shown in Fig. 2.

^c To avoid degeneracy near the 3' end, two pools of primers were designed from some peptides (sequence differences are indicated in boldface).

tfdD gene fragments was performed as described in reference 35. Fragments of *tfdE* and *tfdF* were amplified according to the method of Hoffmann et al. (17).

Sequencing and sequence analysis. For sequencing, PCR products were ligated to pCR2.1 and transformed into *E. coli* TOP10F' by using the TA cloning kit (Invitrogen). Cycle sequencing was performed by using the ABI Prism BigDye Terminator v2.0 cycle sequencing ready reaction kit (PE Applied Biosystems). M13 primers were used for amplifying and sequencing cloned PCR fragments. After amplification with M13 primers and electrophoretic separation, fragments were extracted with the E.Z.N.A gel extraction kit (Pheqab Biotechnologie). Sequencing reactions (total volume, 10 µl) contained 2 µl of premix, 10 pmol primer, and 10 to 50 ng of template DNA. Primer walking on cosmid DNA was performed with primers designed from the sequences of the *rdpA* and *sdpA* PCR products. Sequencing reactions (total volume, 20 µl) contained 6 µl of premix, 20 pmol of primer, and 500 ng of cosmid DNA. After 25 cycles of 96°C for 30 s, 55°C for 15 s, and 60°C for 4 min, the DNA was precipitated by adding 80 µl of H₂O, 10 µl of 3 M sodium acetate (pH 4.6), and 250 µl of ethanol; washed with 70% ethanol; vacuum dried; and redissolved in 20 µl of template suppression reagent (PE Applied Biosystems). Sequencing from cosmid DNA involved an additional purification step prior to the ethanol precipitation by using Centri-Sep spin columns (Princeton Separations). After denaturation at 90°C/2 min the sample was chilled on ice. Electrophoresis, data collection, and analysis were carried out on an ABI Prism 310 genetic analyzer (PE Applied Biosystems). Both strands were sequenced. Sequencing data were analyzed with the Sequence Navigator software, contigs were assembled by using the Autoassembler software (PE Applied Biosystems), and the BLASTn and BLASTx programs (version 2.2.2. [1]) were used to search for similar sequences in nucleotide and protein sequence databases. Sequences were aligned by using the programs CLUSTAL W (51) or MultAlign (7). Phylogenetic analysis was performed by using the protml tool of the ARB package (29) when the maximum-likelihood method was applied and MEGA2.1 (26) when trees were constructed with the neighbor-joining or maximum-parsimony method.

Recombinant pcDNA2.1 DNA was isolated as described elsewhere (2, 3). A Perkin-Elmer 9700 thermocycler was used for cycle sequencing. Sequencing reactions (total volume, 10 µl) contained ca. 300 ng of template, 1.5 µl of ABI Prism BigDye Terminator v1.1 Cycle Sequencing RR-100 mix, 1.2 µl of 5× sequencing buffer, and 2.4 pmol of primer T7 or M13. The sequencing program involved an initial denaturation step (94°C for 60 s), followed by 70 cycles of 96°C for 10 s, 50°C for 5 s, and 60°C for 4 min. Sequencing reactions were precipitated with 40 µl of 76% ethanol for 30 min, washed with 70% ethanol, and resuspended in 10 µl of 0.3 mM EDTA buffer. Electrophoresis, data collection, and analysis were performed by using an ABI Prism 3700 genetic analyzer (PE Applied Biosystems). Automatic sequence assembly was carried out by using PHRED (11, 12), PHRAP (P. Green, unpublished data), and the Autoassembler software. When gaps were closed between contigs, cosmid DNA was used as a template and custom-made oligonucleotides were added as primers. The sequence was edited by using CONSED (15) and annotated by using Vector NTI Advance (InforMax). Similarity searches were carried out as mentioned above.

DNA-DNA hybridization. Screening of the cosmid library was done by replica plating 12 96-well grids onto positively charged nylon membranes (Roche) that

had been placed on selective agar. Cells of strain MC1 (positive control) and *E. coli* XL1-Blue MRF' (pScosPA1) (negative control), as well as strains MH and P230, were smeared onto the nylon membrane placed on LB agar. After overnight growth, DNA was immobilized onto the membranes according to the manufacturer's guidelines.

Genomic DNA of strain MC1 was isolated as described by Sokol et al. (43). The low-copy plasmid pMC1 was isolated with a Nucleobond AX100 kit (Macherey-Nagel). Plasmid and genomic DNA were PstI digested according to standard procedures (40), separated electrophoretically in 0.8% agarose, blotted onto positively charged nylon membrane according to the manufacturer's guidelines, and cross-linked by UV irradiation for 1 min.

Cosmid and plasmid DNA was isolated from 20 ml of LB medium by using the Nucleobond AX100 kit (Macherey-Nagel). Next, 5 µl of cosmid DNA was applied to positively charged nylon membrane and cross-linked by UV irradiation for 1 min. then, 100 µl of plasmid DNA was PstI or EcoRI/XmaI digested according to standard procedures (40), ethanol precipitated, separated, blotted, and UV cross-linked as described above.

PCR fragments were digoxigenin labeled by using the DIG DNA labeling kit (Roche). For hybridization and immunodetection, the DIG Easy Hyb Wash and Block buffer set and the DIG nucleic acid detection kit (Roche) were used according to the manufacturer's guidelines. Hybridizations were performed at 50°C (*tfdC*, *tfdD*, *tfdE*, and *tfdF* probes), 55°C (*rdpA* probe), 60°C (probes against *tfdB*, the *mer* operon, and the *IS91* element), or 62°C (*sdpA* probe). Stripping was carried out according to the manufacturer's instructions.

Nucleotide accession numbers. The nucleotide sequences determined in the present study were deposited in the GenBank, EMBL, and DDBJ databases under accession numbers AY327575, AF516751, and AF516752.

RESULTS

Identification, sequencing, and analysis of dichlorprop-cleaving dioxygenase genes in *D. acidovorans* MC1. Degenerate primers were designed from N-terminal and internal peptide sequences of both enantiospecific dioxygenases of *D. acidovorans* MC1. Primers with a degeneracy near the 3' end proved to be nonspecific; therefore, two pools of primers were synthesized to avoid degeneracy at such positions (Table 1). PCR with the primer pairs rdp_fg-rdp_r (*rdpA*-specific primers) and sdp_fT-sdp_rA (*sdpA*-specific primers) yielded fragments of 339 and 532 bp, respectively, whereas all other primer combinations did not produce specific fragments. The two PCR fragments were cloned and sequenced. The deduced amino acid sequences of both fragments contained sequences at their N and C termini, respectively, that correctly corresponded to the peptide sequences used for designing the prim-

TABLE 2. Sequence identities of the dichlorprop-cleaving enzymes RdpA and SdpA to other group II α KG-dependent dioxygenases

α -KG dioxygenase ^a	Strain, accession no.	% Identities ^b to	
		RdpA	SdpA
TfdA	<i>Ralstonia eutropha</i> , A27082	32/279	37/244
TauD	<i>Pseudomonas aeruginosa</i> , NP_252624	35/279	27/258
AtsK	<i>Pseudomonas putida</i> , AAD31784	31/296	31/296
YSD	<i>Saccharomyces cerevisiae</i> , Q12358	29/279	27/304
PvcB	<i>Pseudomonas aeruginosa</i> , AAC21672	20/298	21/277
SyrI	<i>Pseudomonas syringae</i> , AAB63253	17/323	18/308
CarC	<i>Pectobacterium carotovorum</i> , AAD38231	16/285	17/275
γ -BBH	<i>Pseudomonas</i> sp. strain AK-1, P80193	16/309	17/291
CS2	<i>Streptomyces clavuligerus</i> , AAA26723	16/208	17/275

^a TfdA, 2,4-D/ α KG dioxygenase; TauD, taurine/ α KG dioxygenase; AtsK, putative α KG-dependent alkylsulfatase; YSD, sulfonate/ α KG dioxygenase; PvcB, pyoverdine biosynthesis enzyme; SyrI, syringomycin biosynthesis enzyme I; CarC, carbapenem biosynthesis enzyme C; γ -BBH, γ -butyrobetaine hydroxylase; CS2, clavaminic synthase 2.

^b That is, the percent sequence identity over the total number of amino acids examined.

ers. In screens of a genomic cosmid library of strain MC1 10 of 1,152 clones hybridized with the *rdpA* gene probe. Of these 10, 4 hybridized with the *sdpA* gene probe, but PCR with the *sdpA*-specific primers yielded a product with only 1 of these 4 clones, designated pScos-R5. Both dioxygenase genes were then completely sequenced from this clone. The coding regions of the *R*-dichlorprop and *S*-dichlorprop dioxygenase genes were 888 and 879 bp, respectively. No significant sequence similarities to other known genes were found searching nucleotide databases. However, the deduced amino acid sequences of RdpA and SdpA both shared significant similarities to other α KG-dependent dioxygenases ranging between 37 and 16% (Table 2). RdpA and SdpA shared 26% sequence identities. Furthermore, similarities to a number of putative dioxygenases belonging to the genera *Pseudomonas*, *Yersinia*, *Ralstonia*, *Streptomyces*, *Bordetella*, *Bradyrhizobium*, *Azotobacter*, *Shigella*, *Chromobacterium*, *Burkholderia*, and *Mycobacterium* were found and ranged between 31 and 16%.

The amino acid sequences of both dioxygenases were analyzed on the basis of the alignment shown in Fig. 2. It shows that an internal sequence tentatively aligned to positions 257 to 287 of TfdA by Westendorf et al. (57), comprises positions 85 to 131 of RdpA.

Transposon mutagenesis. Of 959 green fluorescing transposon mutants, 2 were found to carry the transposon insertion in *rdpA*. In clone MC1-T270, the insertion was located after bp 627; in clone MC1-T647, the mini-Tn5 had inserted after bp 814. Both mutants had lost the ability to cleave *R*-dichlorprop but were still able to grow on the *S*-enantiomer.

Organization of *rdpA* and *sdpA* within the genome of *D. acidovorans* MC1. The 25,768-bp sequence of the genomic fragment from strain MC1 containing *rdpA* and *sdpA* in cosmid pScos-R5 is represented in Fig. 3A. The assembly was verified by PCR of selected regions (data not shown). The *rdpA* and *sdpA* genes were separated by 13.6 kb, which mainly comprised an 8.7-kb putative transposon, organized in a way characteristic for the Tn501 subgroup of the Tn3 transposon family (28). It was flanked by 38-bp inverted repeats that were flanked by 5-bp direct repeats generated during insertion. The open reading frames (ORFs) for the putative transposase and resolvase

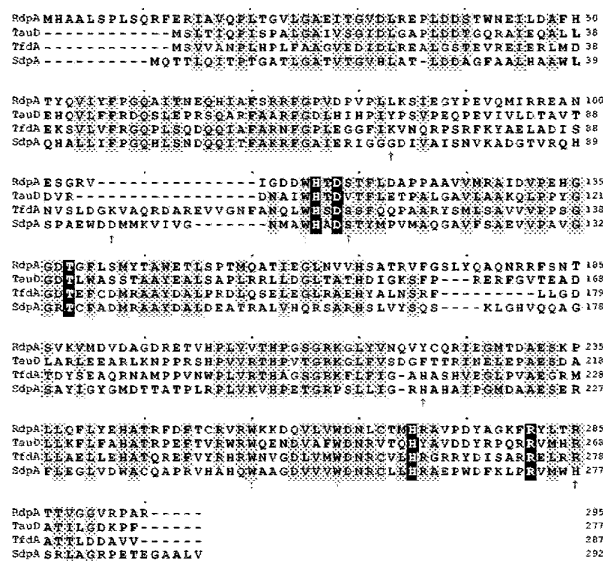


FIG. 2. Sequence alignment of RdpA and SdpA with other group II α KG-dependent dioxygenases. TfdA, *R. eutropha* 2,4-D/ α KG dioxygenase (accession no. A27082); TauD, *P. aeruginosa* taurine/ α KG dioxygenase (accession no. NP_252624). Conserved Fe(II)- and α KG-binding residues are shaded in black; other conserved regions are shaded in gray. Black arrows indicate 2,4-D binding residues in TfdA; gray arrows indicate residues undergoing posttranslational hydroxylation in TauD or TfdA. Amino acid positions numbers are shown to the right of each line. The alignment was created with CLUSTAL W.

genes *tnpA* and *tnpR* had an outward direction with the ORF for *tnpA* proceeding 5 bp into the inverted repeat. The *res* site, at which cointegrate resolution during transposition takes place, was located upstream of *tnpR*. The remaining part of the transposon was occupied by a mercury resistance gene cluster. The *merR* gene had a direction divergent from the *merTP* CADE operon. A putative *urf2M* was located adjacent to the *mer* operon. The putative Tn501 transposon showed 81% nucleotide sequence identity to a putative Tn3 family transposon of *Nitrosomonas europaea* ATCC 19718 (accession no. BX321858.1). The deduced amino acid sequences of the identified ORFs showed between 82 and 97% identity to proteins involved in transposition and mercury resistance (Table 3). The functionality and mobility of this transposon were not tested, but strain MC1 was found to be resistant to 0.1 mM HgCl_2 . Mercury resistance was not lost when dichlorprop-cleaving activity was lost, but these mutants were still able to grow in the presence of 0.1 mM HgCl_2 . In Southern hybridizations the *mer* operon of the plasmid was localized on a 4.4-kb fragment of pMC1 (Fig. 4C). This copy was deleted in the plasmid of the mutant MC1-DP⁻. However, in hybridizations against genomic DNA, another signal at 2.8 kb was detected that was present in the wild-type strain as well as in strain MC1-DP⁻ (data not shown), suggesting that strain MC1 carries a second copy of the *mer* operon on the chromosome that is not lost when the dichlorprop-cleaving ability is lost. It is possible that the chromosomal copy of the *mer* operon confers mercury resistance to strains MC1 and MC1-DP⁻, whereas the copy on the plasmid may lack functionality.

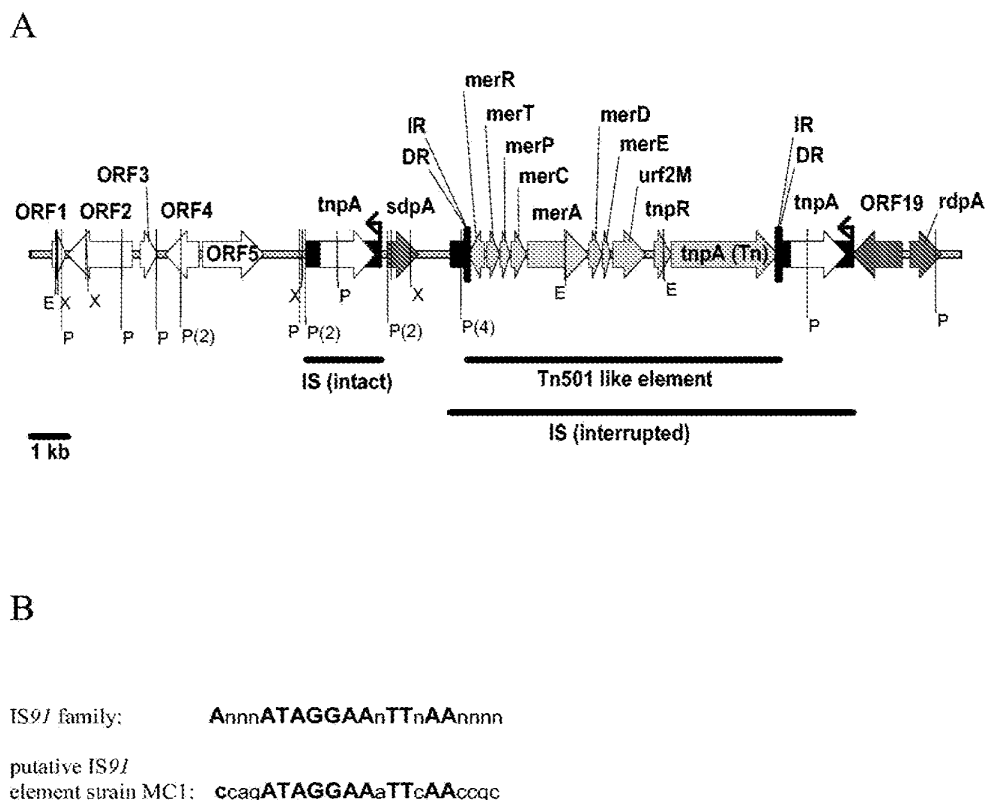


FIG. 3. Organization of *rdpA* and *sdpA* within the genome of strain MC1. (A) Genetic map of the genomic fragment containing *rdpA* and *sdpA*. For ORFs showing a high degree of similarity to other genes (>80% identity of the deduced amino acid sequence) the respective names are given. The gene designations are as follows: *tnpA/tnpA*(Tn), putative transposases; *sdpA*, *S*-dichlorprop/ α -ketoglutarate dioxygenase; *merR*, putative mercury resistance operon transcriptional regulator; *merT*, *merP* and *merC*, putative mercury ion transport genes; *merA*, putative mercury reductase; *merD*, putative secondary regulator; *merE* and *urf2M*, conserved ORF of unknown function; *tnpR*, putative site-specific resolvase; and *rdpA*, *R*-dichlorprop/ α -ketoglutarate dioxygenase. ORF1 through ORF5 and ORF19 show significant similarities to genes encoding the proteins as follows: ORF1, carboxymuconolactone decarboxylase; ORF2, potassium/proton antiporter; ORF3, bacterioferritin; ORF4, LysR type regulator; ORF5, dihydroxyacid dehydratase; and ORF19, MFS transporter. The positions of the following features are indicated: IR, 38-bp inverted repeat flanking the Tn501-like transposon, DR, 5-bp direct repeat. Two putative IS91-like elements are shown as black boxes; the positions of the *oriIS* are indicated as arrows above the sequence. The right IS91-like element is interrupted by a Tn501-like transposon. Its left partial sequence is not drawn to scale. Single letters denote the following restriction sites: E, EcoRI; P, PstI; and X, XmaI. In the case of multiple PstI restriction sites within less than 100 bp, only the most left site is given and the number of sites is indicated in brackets. (B) Comparison of the sequence at the right end of the putative IS elements with the IS91 family *oriIS* consensus sequence. Conserved nucleotides are shown in bold uppercase letters.

Furthermore, two copies of a putative IS element resembling the IS91/IS801 family of insertion elements were identified. Members of this family of IS elements do not contain inverted repeats and do not create duplications of the target sequence (14). Insertion of this family of elements is site specific, and replication utilizes the rolling circle mechanism; therefore, an *oriIS*, the origin of replication, and a *terIS*, the termination sequence, are found at either end of these IS elements (14). The two copies of the putative IS elements in strain MC1 were essentially identical except for a 9-bp deletion and a 47-bp deletion in the left, intact copy. The element next to ORF19 was interrupted by the Tn501-like element (Fig. 3A). The elements were 2,037 bp (intact copy) and 2,084 bp (interrupted copy) and contained an IS801-like transposase gene showing 99% sequence identity to the homologues on the atrazine catabolic plasmid pADP-1 (Table 3 [32]) and a transposase associated with chlorobenzene degradation genes in *Ralstonia* sp. strain JS705 (37). At the right end of both elements a

sequence was identified, that matched the consensus sequence for origin of replication *oriIS* of this group of insertion elements (49) in all but the first nucleotide (Fig. 3B). Neither the termination sequence *terIS* nor the tetramers GTTC/CTTG for which IS91 family elements show site specificity were detected.

ORF19, located upstream of *rdpA* showed significant similarities to aromatic compound major facilitator superfamily (MFS) transporters. A 29% identity to the 2,4-D transporters CadK of *Bradyrhizobium* sp. strain HW13 and TfdK of *Achromobacter xylosoxidans* subsp. *denitrificans* and *R. eutropha* JMP134 was detected. A putative Shine-Dalgarno sequence was found 7 bp upstream of the start codon.

Another five ORFs, ORF1 to ORF5, were identified at the left end side of the insert.

Localization of *rdpA* and *sdpA* within the genome of *D. acidovorans* MC1 and comparison of the wild-type plasmid pMC1 with that of the mutant MC1-DP⁻. Southern hybridizations showed that both *rdpA* and *sdpA* were located on the

TABLE 3. Localization and predicted function of ORFs in the genomic region of strain MC1 containing *rdpA* and *sdpA*

ORF	Coordinates (bp)	Coding strand ^a	Function of closest match	Source microorganism	% Amino acid identity ^b	No. of amino acids MC1/match ^c	Accession no.
1	574-966	d	Carboxymuconolactone decarboxylase family protein	<i>Shewanella oneidensis</i> MR-1	65 (81/124)	130/128	AAN53170.1
2	1006-2799	c	Glutathione-regulated potassium-proton antiporter	<i>Pseudomonas putida</i> KT2440	69 (385/556)	597/581	AE016523.1
3	3018-3494	d	Putative bacterioferritin	<i>Deiftia acidovorans</i>	70 (112/158)	158/158	CAC83620.1
4	3701-4642	c	Hypothetical protein, similar to LysR-type regulators	<i>Ralstonia metallidurans</i>	51 (161/314)	313/360	ZP_00022545.1
5	4745-6438	d	Putative dihydroxy acid dehydratase	<i>Ralstonia solanacearum</i>	76 (420/555)	564/557	CAD16119.1
6	7981-9528	d	Putative transposase, similar to IS801 transposase	<i>Pseudomonas</i> sp. strain ADP	99 (512/515)	515/515	AAK50272.1
7	9836-10714	d	2,4-D dioxygenase (TfdA)	<i>Ralstonia eutropha</i> JMP134	37 (92/249)	292/287	AZ7082
8	12107-12541	c	Mercury resistance regulatory protein (MerR)	<i>Enterobacter aerogenes</i>	97 (140/144)	144/144	CAA83896.1
9	12613-12963	d	Mercury uptake protein (MerT)	<i>Alcaligenes</i> sp.	96 (112/116)	116/116	CAA70196.1
10	12977-13251	d	Mercury uptake protein (MerP)	<i>Alcaligenes</i> sp.	94 (86/91)	91/91	CAA70197.1
11	13279-13701	d	Mercury uptake protein (MerC)	<i>Nitrosomonas europaea</i> ATCC 19718	86 (121/140)	140/140	CAD84751.1
12	13741-15426	d	Mercury ion reductase (MerA)	<i>Klebsiella pneumoniae</i>	88 (494/561)	561/561	AAM44226.1
13	15444-15809	d	Mercury resistance secondary regulatory protein (MerD)	<i>Pseudomonas</i> sp. strain ADP	95 (115/121)	121/121	AAK50284.1
14	15806-16042	d	Putative protein (MerE)	<i>Pseudomonas</i> sp. strain ADP	91 (71/78)	78/78	AAK50283.1
15	16093-17028	d	Putative protein, similar to Urf2M	<i>Ralstonia metallidurans</i>	88 (274/310)	311/329	CAD97548.1
16	17245-17718	d	Resolvase (TnpR)	<i>Nitrosomonas europaea</i> ATCC 19718	98 (154/157)	158/186	CAD84747.1
17	17721-20690	d	Tn3 family transposase (TnpA)	<i>Nitrosomonas europaea</i> ATCC 19718	95 (945/989)	989/989	CAD84746.1
18	21024-22571	d	Putative transposase, similar to IS801 transposase	<i>Pseudomonas</i> sp. strain ADP	99 (511/515)	515/515	AAK50272.1
19	22752-24137	c	Putative aromatic compound MFS transporter	<i>Pseudomonas putida</i> KT2440	73 (318/433)	461/450	AAN69258.1
20	24330-25217	d	Taurine dioxygenase (TauD)	<i>Pseudomonas aeruginosa</i> PA01	35 (100/284)	295/279	NP_252624

^a c, complementary strand; d, direct strand.^b Values in parentheses refer to numbers of identical amino acids over the total number examined.^c Number of amino acids in strain MC1 ORF/number in the closest match.

plasmid pMC1 (Fig. 4A). Distinct signals of the expected fragment sizes of 3.5 kb (*rdpA*) and 1.9 kb (*sdpA*) were observed with plasmid DNA (Fig. 4A, lane 1). Much fainter signals of the same fragment sizes were obtained with the genomic DNA, making visible the plasmid content of this. Loss of dichlorprop cleavage in strain MC1-DP⁻ was associated with a deletion of both dioxygenase genes from pMC1-DP⁻ (Fig. 4B). Furthermore, the *mer* operon and most of the numerous copies of the IS91-like elements were also deleted in the mutant MC1-DP⁻ (Fig. 4C). In contrast, the lower pathway genes *tfdB-F* remained unaffected (data not shown).

Screening for *rdpA* and *sdpA* in other dichlorprop-degrading bacteria. Southern hybridizations with *rdpA* and *sdpA* probes showed that neither *S. herbicidovorans* MH nor *Rhodospirillum* sp. strain P230 carried *sdpA*-like genes, but both strains gave signals with the *rdpA* gene probe (data not shown). The genes were PCR amplified and sequenced. The *rdpA* gene of strain P230 was identical to that of strain MC1; the one of strain MH contained one neutral base substitution.

DISCUSSION

Two novel genes encoding the enantiospecific dichlorprop and mecoprop cleaving dioxygenases RdpA and SdpA in *D. acidovorans* MC1 were identified. The characterization of genes shown to be involved in the cleavage of these chiral herbicides has not previously been reported. To date, *tfdA* genes or genes closely related in sequence to *tfdA* were proposed to be involved in *R*-dichlorprop cleavage in *Alcaligenes denitrificans* (39). This suggestion was based on the observations that a *tfdA* gene is present in *Alcaligenes denitrificans* and that dichlorprop cleavage is catalyzed by an α KG-dependent dioxygenase in this strain as well as in *S. herbicidovorans* MH (38). However, it was not supported by *tfdA* expression studies or amino acid sequences of the *R*-dichlorprop cleaving dioxygenase. The *rdpA* and *sdpA* genes of strain MC1 showed no significant nucleotide similarities to *tfdA*. However, the significant degree of identity of their deduced amino acid sequences to group II α KG-dependent dioxygenases and the distribution of these identities throughout the whole of the primary sequence (Fig. 2) supported the view that RdpA and SdpA are homologous to dioxygenases of this family. The functionally important motif HX(D/E)X₂₃₋₂₆(T/S)X₁₁₄₋₁₈₃HX₁₀₋₁₃R/K, which is conserved throughout group II α KG-dependent dioxygenases (18), is present in both RdpA and SdpA. In TfdA of *R. eutropha* it includes the three Fe(II)-binding residues His114, Asp116, and His263, and the two α KG-binding residues Thr141 and Arg274 (Fig. 2). All of these residues were conserved in RdpA and SdpA, representing the putative Fe(II) and α KG-binding residues in these enzymes. Arg278, His214, Ser117, Lys95, and Lys71 of TfdA are known to interact with 2,4-D (8, 10). Ser117 homologues were identified in both dichlorprop-cleaving enzymes. A homologue for Arg278 was present only in RdpA, one for His214 was found in SdpA. The tryptophan residues Trp126, Trp238, and Trp246 undergoing posttranslational hydroxylation in TauD and Trp113 undergoing posttranslational hydroxylation in TfdA form a hydrophobic cluster around the active site (10). In RdpA and SdpA all of these residues were conserved or substituted by other hydrophobic amino acids. The basis of the enantiospecificity of

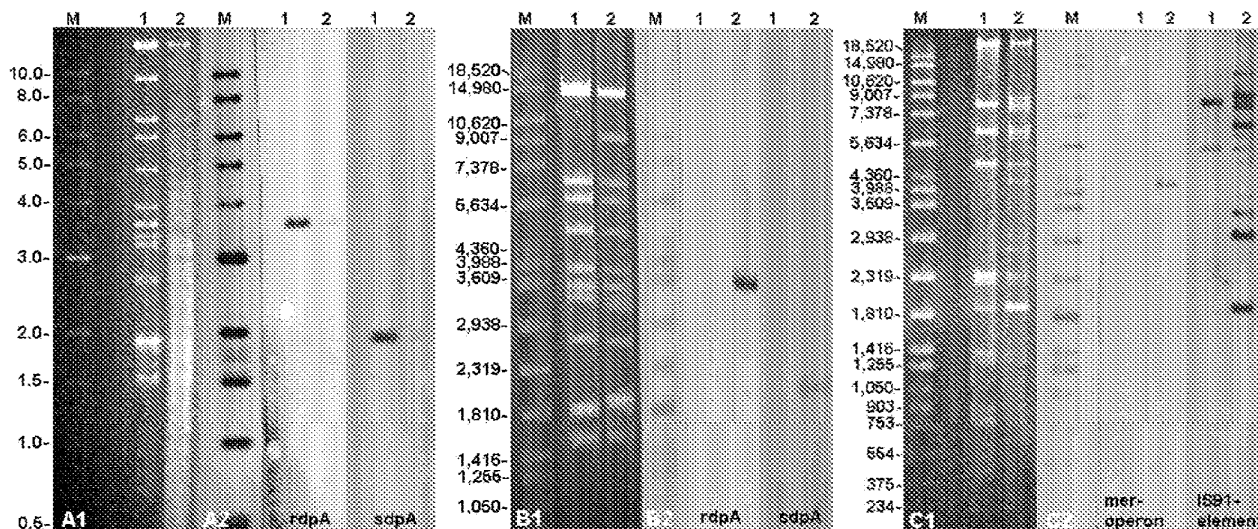


FIG. 4. Localization of *rdpA* and *sdpA* within the genome of *D. acidovorans* MC1 and comparison of plasmid pMC1 with pMC1-DP⁻, the plasmid of mutant MC1-DP⁻. (A) Localization of *rdpA* and *sdpA* within the genome of strain MC1. Plasmid DNA (lane 1) and genomic DNA (lane 2) was digested with PstI, separated electrophoretically in 0.8% agarose (A1), and hybridized (A2). Gene probes are given at the bottom. (B and C) Comparison of plasmid pMC1 of the wild-type strain of MC1 (lane 2) with pMC1-DP⁻, the plasmid of the mutant unable to cleave dichlorprop (lane 1). PstI-digested plasmid DNA was separated electrophoretically in 0.8% agarose (B1) and hybridized against *rdpA* and *sdpA* gene probes (B2). EcoRI/XmaI-digested plasmid DNA was separated electrophoretically in 0.8% agarose (C1) and hybridized against probes for the *mer* operon and the IS91 element (C2).

the dichlorprop-cleaving enzymes is not known. It is known that the tertiary structure of homologous proteins showing at least 20% identity of amino acid sequence is conserved (41). Among group II α KG dioxygenases a high degree of structural conservation was observed between clavaminic synthase and TauD, which share as little as 7.7% primary sequence identity (10). It is therefore likely that 2,4-D and dichlorprop-cleaving enzymes are structurally very similar and that substrate specificity is determined by only a few amino acids. Site-specific mutagenesis studies are needed to reveal the molecular basis of the substrate specificity and stereospecificity of RdpA and SdpA.

Phylogenetic trees calculated by different methods (data not shown) consistently grouped RdpA in one clade with TauD enzymes, but the assignment of SdpA could not be determined precisely. The primary sequence of SdpA most closely resembled that of TfdA and, like this dioxygenase, SdpA is characterized by a relatively broad substrate specificity (34). TfdA has greatest activity with 2,4-D but shows some activity toward *S*-mecoprop (38). SdpA shows greatest activity with the *S*-enantiomers of mecoprop and dichlorprop but has some activity toward 2,4-D (34). These observations suggested that SdpA is most closely related to 2,4-D-cleaving enzymes, which was also consistent with the position of SdpA in trees constructed without an outgroup. However, when PvcB was used as an outgroup SdpA was represented as a sister group of both the TfdA/TfdA α and the RdpA/TauD clades. At present, the database is too small to determine more precisely the phylogenetic relationship between SdpA and other members of the α KG dioxygenase family.

Transposon mutagenesis inactivation of *rdpA* indicated that it was a single-copy gene and gave a further proof of its func-

tionality and role in strain MC1, besides previous characterization of RdpA and SdpA (34, 57, 58).

An *rdpA* gene identical to that of strain MC1 was identified in *Rhodospirillum rubrum* sp. strain P230, and one nearly identical was found in *S. herbicidovorans* MH, but neither of these strains carried *sdpA* genes, as indicated by Southern hybridizations allowing ca. 20% mismatches. The *rdpA* genes could be involved in *R*-dichlorprop degradation in strains P230 and MH. Further investigations are necessary to study their role to determine whether *rdpA* genes are generally more widely distributed than *sdpA* genes, as these limited data suggest, and to identify the genes encoding the *S*-dichlorprop-cleaving dioxygenases of strains MH and P230.

In *S. herbicidovorans* MH, three transport proteins specific for 2,4-D, *R*-dichlorprop, and *S*-dichlorprop were identified (60). In strain MC1, ORF19 encodes a putative transport protein (Fig. 4 and Table 3). It was in close proximity to *rdpA*, and the significant similarity of the deduced protein sequence to TfdK and CadK suggested an involvement in *R*-dichlorprop transport. No other potential transporters were detected. Likewise, no genes encoding transcriptional regulators of dichlorprop cleavage were identified. This is in accordance with previous studies which found *rdpA* and *sdpA* to be constitutively expressed (36). ORF4, which shows significant similarities to LysR-type transcriptional regulators, probably regulates the expression of ORF5 rather than that of *sdpA* or *rdpA*.

The ability to degrade dichlorprop in *D. acidovorans* MC1 is spontaneously and irreversibly lost at high frequencies under nonselective conditions, whereas the degradation of dichlorophenol is a stable trait (36). This indicated that the ability to cleave the phenoxyalkanoate herbicides is unstable in strain MC1, which would be consistent with recent acquisition of the

rdpA and *sdpA* genes. The localization and organization of the initial step in dichlorprop degradation imply that its evolution and recruitment took place independently of the lower pathway. Cases and de Lorenzo (5) postulated that degradative pathways for substrates only very recently found in the environment are often poorly regulated, probably reflecting an early step in the optimization of the corresponding metabolic route, and that biodegradative operons evolve from constitutive expression to substrate-responsive and metabolically controlled transcription. The evolution of the dichlorprop cleavage pathway in strain MC1 is an example supporting this hypothesis, since *rdpA* and *sdpA* are constitutively expressed, whereas the consecutive degradation of the intermediate dichlorophenol is inducible (36).

Previously, the loss of genes involved in etherolytic cleavage of dichlorprop was hypothesized to be caused by a deletion within plasmid pMC1, which occurs concomitantly with loss of dichlorprop cleavage (36). In the present study, both dioxygenase genes were shown to be located on the plasmid and to be deleted in the MC1-DP⁻ mutant, proving this hypothesis to be correct. Moreover, the *mer* operon was deleted, and the copy number of the *IS91*-like element was found to be considerably reduced. This suggests that recombination between *IS91* elements could lead to the deletion of one or both dioxygenase genes and the Tn501-like transposon. Although *IS91*-like elements do not cause deletion of regions adjacent to them, a deletion of a genomic fragment flanked by *IS91*-like elements was observed in *Shigella flexneri* (52). In strain MC1, recombination between the interrupted *IS* element and the intact *IS* copy downstream of ORF5 would lead to the deletion of the region between the elements, including *sdpA*. Likewise, the *IS* elements may have played a role in the acquisition of dichlorprop cleavage in strain MC1. In *Ralstonia* sp. strain JS705 the *clc* genes are flanked by an intact and a partial copy of an insertion element highly similar to the ones identified in strain MC1. These *IS* elements were proposed to have captured the *clc* genes and made possible the gene transfer from *Ralstonia* sp. strain JS745 to strain JS705 (37). Characterization of the region downstream of *rdpA* and comparison of the MC1-DP⁻ mutant genotype with the MC1 wild-type will further the understanding of processes having lead to the acquisition and causing instability of the degradative trait.

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DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
1) X	DATABASE EMBL [Online] 3 March 2004 (2004-03-03), "Sphingomonas herbicidovorans tnpA gene for transposase and rdpA gene for (R)-2-(2,4-dichlorophenoxy)propionate, 2-oxoglutarate dioxygenase" XP002474325 ✓ retrieved from EBI accession no. EMBL:AJ628859 Database accession no. AJ628859 * abstract * & KOHLER H P E: "Sphingomonas herbicidovorans MH: A versatile phenoxyalkanoic acid herbicide degrader" JOURNAL OF INDUSTRIAL MICROBIOLOGY AND BIOTECHNOLOGY, vol. 23, no. 4-5, October 1999 (1999-10), pages 336-340, ISSN: 1367-5435	39, 40, 42	INV. C12N15/53 C12N15/82 A01H5/00 A01H5/10
2) X	DATABASE EMBL [Online] 6 August 2002 (2002-08-06), "Rhodoferax sp. P230 R-2,4-dichlorophenoxypropionate dioxygenase (rdpA) gene, complete cds." XP002474326 ✓ retrieved from EBI accession no. EMBL:AF516751 Database accession no. AF516751 * abstract *	39, 40, 42	TECHNICAL FIELDS SEARCHED (IPC) C12N A01H
fehlt 3) X	& DATABASE EMBL [Online] 6 August 2002 (2002-08-06), "Sphingobium herbicidovorans R-2,4-dichlorophenoxypropionate dioxygenase (rdpA) gene, complete cds." XP002474327 retrieved from EBI accession no. EMBL:AF516752 Database accession no. AF516752 * abstract * -/-	39, 40, 42	
The supplementary search report has been based on the last set of claims valid and available at the start of the search.			
Place of search The Hague		Date of completion of the search 28 March 2008	Examiner Oderwald, Harald
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

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EPO FORM 1503 03 82 (P04CC04)



European Patent
Office

**SUPPLEMENTARY
EUROPEAN SEARCH REPORT**

Application Number
EP 05 77 1746

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (IPC)
	& SCHLEINITZ KATHLEEN M ET AL: "Localization and characterization of two novel genes encoding stereospecific dioxygenases catalyzing 2(2,4-dichlorophenoxy)propionate cleavage in <i>Delftia acidovorans</i> MC1" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 70, no. 9, September 2004 (2004-09), pages 5357-5365, ISSN: 0099-2240		
4) A	WO 98/02562 A (RHONE-POULENC AGROCHIMIE [FR]; PALLETT KEN [GB]; DEROSE RICHARD [FR];) 22 January 1998 (1998-01-22) * the whole document *	1,5,11, 35,44, 47, 51-53, 57,58	
5) A	WO 03/034813 A (NASA [US]) 1 May 2003 (2003-05-01) * the whole document *	1,5,11, 35,44, 47, 51-53, 57,58	TECHNICAL FIELDS SEARCHED (IPC)
The supplementary search report has been based on the last set of claims valid and available at the start of the search.			
Place of search The Hague		Date of completion of the search 28 March 2008	Examiner Oderwald, Harald
CATEGORY OF CITED DOCUMENTS X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document			

1
EPO FORM 1503 03/82 (P04C04)

**ANNEX TO THE EUROPEAN SEARCH REPORT
ON EUROPEAN PATENT APPLICATION NO.**

EP 05 77 1746

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

28-03-2008

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
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			AU 3625997 A	09-02-1998
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			CA 2464426 A1	01-05-2003
			US 2003154507 A1	14-08-2003

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82

Electronic Acknowledgement Receipt	
EFS ID:	3604714
Application Number:	11587893
International Application Number:	
Confirmation Number:	7868
Title of Invention:	Novel Herbicide Resistance Genes
First Named Inventor/Applicant Name:	Terry Wright
Customer Number:	23557
Filer:	Jay M. Sanders/Mary Calderwood
Filer Authorized By:	Jay M. Sanders
Attorney Docket Number:	DAS-115XC1
Receipt Date:	11-JUL-2008
Filing Date:	
Time Stamp:	16:35:15
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no
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File Listing:

Document Number	Document Description	File Name	File Size(Bytes) /Message Digest	Multi Part /.zip	Pages (if appl.)
1	Information Disclosure Statement Letter	IDS-suppltr.pdf	58980 0fc41f7c9848b56f6782d01700361c70870a111e	no	2

Warnings:

Information:

2	Information Disclosure Statement (IDS) Filed	IDS-supp-Form.pdf	105701	no	2
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Warnings:					
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3	Foreign Reference	WO98002562.pdf	2214576	no	49
			055877535fac7e9369245015de8f8a5166a2a981		
Warnings:					
Information:					
4	Foreign Reference	WO03034813.pdf	2492350	no	80
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5	NPL Documents	XP002474325.pdf	332026	no	2
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6	NPL Documents	Kohler.pdf	75590	no	5
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7	NPL Documents	XP002474326.pdf	206463	no	2
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9	NPL Documents	Schleintz.pdf	1014686	no	9
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10	NPL Documents	EPO-Suppl-Search-Report.pdf	271511	no	3
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Warnings:	
Information:	
Total Files Size (in bytes):	6970531
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.	

I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademark Office on Aug. 21, 2008.


Jay M. Sanders, Patent Attorney

Patent Application
Docket No. DAS-115XC1
Serial No. 11/587,893

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Terry R. Wright, Justin M. Lira, Donald J. Merlo, Nicole L. Arnold
Serial No. : 11/587,893
Filed : October 27, 2006
Conf. No. : 7868
For : Novel Herbicide Resistance Genes

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

TRANSMITTAL LETTER

Sir:

Per MPEP § 605.04(c), a petition under 37 C.F.R. § 1.182 accompanies this submission.

A Notification of Missing Requirements dated June 30, 2008, was received from the Patent and Trademark Office advising that the current oath or declaration does not comply with 37 CFR 1.497(a) and (b) in that there is an inventor name change without supporting documentation.

The above-referenced patent application was filed on May 2, 2005, as a national stage application of International patent Application No. PCT/US2005/014737. At that time, the fourth inventor was using her maiden name of "Nicole Hopkins."

This fourth inventor subsequently adopted her married name of "Nicole L. Arnold" for the execution of all documents in the subject application. Accordingly, an executed Declaration under 37 CFR 1.63 was submitted to the Patent Office on May 20, 2008.

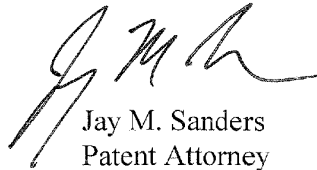
Attached is a Declaration by this inventor attesting to her name change and providing supporting additional details.

J:\DAS\115XC1\PTO\TransLtr-DecNameCng-newDecls.doc/DNB/sl

Applicants respectfully request that the U.S. Patent Office databases regarding the subject application be updated to indicate that the fourth inventor is now known as "Nicole L. Arnold" (replacing the prior name of "Nicole Hopkins"; Nicole Arnold and Nicole Hopkins are one and the same person).

The surcharge of \$130.00 was already paid upon the submission of the Declarations on May 20, 2008. However, if further fees are required by the subject paper, the Commissioner is hereby authorized to charge any additional fees that may be required to Deposit Account No. 19-0065.

Respectfully submitted,

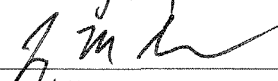


Jay M. Sanders
Patent Attorney
Registration No. 39,355
Telephone No.: (352) 375-8100
Facsimile No.: (352) 372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

JMS/sl

Attachments: Petition under 37 C.F.R. § 1.182
Declaration of Nicole L. Arnold

I hereby certify that this correspondence is being electronically filed in the United States Patent and Trademarks Office on Aug. 21, 2008.



Jay M. Sanders, Patent Attorney

PETITION UNDER 37 C.F.R. § 1.182
Patent Application
Docket No. DAS-115XC1
Serial No. 11/587,893

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Terry R. Wright, Justin M. Lira, Donald J. Merlo, Nicole L. Arnold
Serial No. : 11/587,893
Filed : October 27, 2006
Conf. No. : 7868
For : Novel Herbicide Resistance Genes

Office of Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

PETITION UNDER 37 C.F.R. § 1.182

Sir:

The above-referenced patent application was filed on May 2, 2005, as a national stage application of International patent Application No. PCT/US2005/014737. At that time, the fourth inventor was using her maiden name of "Nicole Hopkins."

This fourth inventor subsequently adopted her married name of "Nicole L. Arnold" for the execution of all documents in the subject application. Accordingly, an executed Declaration under 37 CFR 1.63 was submitted to the Patent Office on May 20, 2008.

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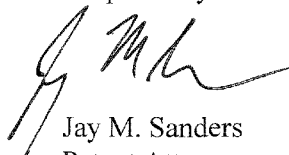
Attached is a Declaration by this inventor attesting to her name change and providing

additional details.

Applicants respectfully request that the U.S. Patent Office databases regarding the subject application be updated to indicate that the fourth inventor is now known as "Nicole L. Arnold" (replacing the prior name of "Nicole Hopkins"; Nicole Arnold and Nicole Hopkins are one and the same person).

Please charge the surcharge of \$130.00 required by 37 C.F.R. § 1.17(i) to Deposit Account No. 19-0065. The Commissioner is hereby authorized to charge any additional fees which may be required to Deposit Account No. 19-0065.

Respectfully submitted,



Jay M. Sanders
Patent Attorney
Registration No. 39,355
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

JMS/jb

Attachment: Declaration of Nicole L. Arnold

DECLARATION
Docket No. DAS-115XC1
Serial No. 11/587,893

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Terry R. Wright, Justin M. Lira, Donald J. Merlo, Nicole L. Arnold
Serial No. : 11/587,893
Filed : October 27, 2006
Conf. No. : 7868
For : Novel Herbicide Resistance Genes

Office of Petitions
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF NICOLE L. ARNOLD

Sir:

I, Nicole L. Arnold hereby declare:

THAT, I was using my maiden name of Nicole Hopkins at the time International patent Application No. PCT/US2005/014737, was filed on May 2, 2005; and

THAT, I have subsequently adopted my married name of Nicole L. Arnold for the execution of all documents related to the subject application;

THAT, I became married on Oct. 15, 2005.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the patent.

Further declarant sayeth not.

Nicole L. Arnold
Nicole L. Arnold (current name)

28 July 2008
Date

Nicole Hopkins
Nicole Hopkins (previous name)

28 July 2008
Date

Electronic Patent Application Fee Transmittal				
Application Number:		11587893		
Filing Date:				
Title of Invention:		Novel Herbicide Resistance Genes		
First Named Inventor/Applicant Name:		Terry Wright		
Filer:		Jay M. Sanders/Sherry Loke		
Attorney Docket Number:		DAS-115XC1		
Filed as Large Entity				
U.S. National Stage under 35 USC 371 Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Oath/decl > 30 months from priority date	1617	1	130	130
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				130

Electronic Acknowledgement Receipt	
EFS ID:	3819758
Application Number:	11587893
International Application Number:	
Confirmation Number:	7868
Title of Invention:	Novel Herbicide Resistance Genes
First Named Inventor/Applicant Name:	Terry Wright
Customer Number:	23557
Filer:	Jay M. Sanders/Sherry Loke
Filer Authorized By:	Jay M. Sanders
Attorney Docket Number:	DAS-115XC1
Receipt Date:	21-AUG-2008
Filing Date:	
Time Stamp:	16:48:20
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 130
RAM confirmation Number	1996
Deposit Account	190065
Authorized User	

The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:

Charge any Additional Fees required under 37 C.F.R. 1.492 (National application filing, search, and examination fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.17 (Patent application and reexamination processing fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.19 (Document supply fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.20 (Post Issuance fees)

Charge any Additional Fees required under 37 C.F.R. Section 1.21 (Miscellaneous fees and charges)

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Response to Pre-Exam Formalities Notice	TransLtr-DecNameCng.pdf	305338 525fd1edf15242a21db19b99ae19ce8016871870	no	6

Warnings:

Information:

2	Fee Worksheet (PTO-06)	fee-info.pdf	30209 98300084b738bc612843d29d3e78b48077d5a3e9	no	2
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Warnings:

Information:

Total Files Size (in bytes):	335547
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This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



25 NOV 2008

Commissioner for Patents
United States Patent and Trademark Office
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Alexandria, VA 22313-1450
www.uspto.gov

SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO BOX 142950
GAINESVILLE FL 32614-2950

In re Application of :
WRIGHT et al. :
Application No.: 11/587,893 : DECISION ON PETITION
PCT No.: PCT/US05/14737 :
Int. Filing Date: 02 May 2005 : UNDER 37 CFR 1.182
Priority Date: 30 April 2004 :
Attorney Docket No.: DAS-115XC1 :
For: NOVEL HERBICIDE RESISTANCE GENES :

This is a decision on applicant's a Petition under 37 CFR 1.182 filed on 21 August 2008 in the United States Patent and Trademark Office.

BACKGROUND

On 27 October 2006, applicant filed a transmittal letter concerning a filing under 35 U.S.C. 371 accompanied by the basic national fee. On 22 May 2008, applicant filed an executed declaration in the above referenced application.

On 30 June 2008, a Notification of Missing Requirements was mailed to applicants indicating that an oath or declaration, in compliance with 37 CFR 1.497(a) & (b) and the surcharge for filing the oath or declaration after the thirty month period, was required. Applicant was advised that the declaration filed on 22 May 2008 did not comply with 37 CFR 1.497(a) and (b) as inventor's name had changed without documentation.

On 21 August 2008, applicant filed a petition under 37 CFR 1.182 to correct the inventor's name.

DISCUSSION

A review of the application file reveals that the given name of the applicant as listed on the published international application is "Nicole Hopkins". The declaration, filed on 22 May

2008, was signed by "Nicole L. Arnold" and was unacceptable.

Applicant now submits declaration by the inventor Nicole Hopkins indicating that her name changed due to her marriage, providing sufficient evidence of her name change.

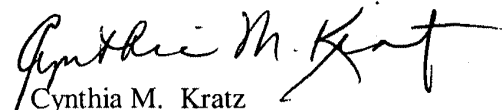
The executed declarations are in compliance with 37 CFR 1.497(a) and (b) as the declaration names the same inventive entity identified in the published international application and state the citizenship, residency and mailing addresses of the named inventors. The declaration satisfies the requirements of 35 U.S.C. 371 (c)(4) for entrance into the national stage in the United States.

CONCLUSION

For the above reasons, the declaration submitted on 22 May 2008 now meets the requirements of 37 CFR 1.497(a) and (b) and is acceptable.

The petition under 37 CFR 1.182 to change the respective inventor's name to Nicole L. Arnold is GRANTED.

The application will be forwarded to the United States Designated/Elected Office for further processing. The 35 U.S.C. 371(c)(1), (c)(2) and (c)(4) date is **22 May 2008**.


Cynthia M. Kratz
Attorney Advisor
Office of PCT Legal Administration

Telephone: (571) 272-3286

Facsimile: (571) 273-0459



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

U.S. APPLICATION NUMBER NO.	FIRST NAMED APPLICANT	ATTY. DOCKET NO.
11/587,893	Terry R. Wright	DAS-115XC1
23557 SALIWANCHIK LLOYD & SALIWANCHIK A PROFESSIONAL ASSOCIATION PO BOX 142950 GAINESVILLE, FL 32614-2950		INTERNATIONAL APPLICATION NO. PCT/US2005/014737
		L.A. FILING DATE 05/02/2005
		PRIORITY DATE 04/30/2004

CONFIRMATION NO. 7868
371 ACCEPTANCE LETTER



Date Mailed: 12/23/2008

NOTICE OF ACCEPTANCE OF APPLICATION UNDER 35 U.S.C 371 AND 37 CFR 1.495

The applicant is hereby advised that the United States Patent and Trademark Office in its capacity as a Designated / Elected Office (37 CFR 1.495), has determined that the above identified international application has met the requirements of 35 U.S.C. 371, and is ACCEPTED for national patentability examination in the United States Patent and Trademark Office.

The United States Application Number assigned to the application is shown above and the relevant dates are:

<u>05/22/2008</u>	<u>08/21/2008</u>
DATE OF RECEIPT OF 35 U.S.C. 371(c)(1), (c)(2) and (c)(4) REQUIREMENTS	DATE OF COMPLETION OF ALL 35 U.S.C. 371 REQUIREMENTS

A Filing Receipt (PTO-103X) will be issued for the present application in due course. **THE DATE APPEARING ON THE FILING RECEIPT AS THE " FILING DATE" IS THE DATE ON WHICH THE LAST OF THE 35 U.S.C. 371 (c)(1), (c)(2) and (c)(4) REQUIREMENTS HAS BEEN RECEIVED IN THE OFFICE. THIS DATE IS SHOWN ABOVE.** The filing date of the above identified application is the international filing date of the international application (Article 11(3) and 35 U.S.C. 363). Once the Filing Receipt has been received, send all correspondence to the Group Art Unit designated thereon.

The following items have been received:

- Copy of the International Application filed on 10/27/2006
- Copy of the International Search Report filed on 10/27/2006
- Copy of IPE Report filed on 10/27/2006
- Preliminary Amendments filed on 10/27/2006
- Information Disclosure Statements filed on 07/11/2008
- Biochemical Sequence Diskette filed on 10/27/2006
- Oath or Declaration filed on 05/22/2008
- Biochemical Sequence Listing filed on 10/27/2006
- Request for Immediate Examination filed on 10/27/2006
- U.S. Basic National Fees filed on 10/27/2006
- Assignment filed on 12/17/2008
- Priority Documents filed on 10/27/2006
- Power of Attorney filed on 05/22/2008
- Specification filed on 10/27/2006
- Claims filed on 10/27/2006
- Abstracts filed on 10/27/2006

- Drawings filed on 10/27/2006
- Paper nucleotide sequence listings filed on 10/27/2006
- Paper Computer Listings filed on 11/08/2006

Applicant is reminded that any communications to the United States Patent and Trademark Office must be mailed to the address given in the heading and include the U.S. application no. shown above (37 CFR 1.5)

BARBARA A CAMPBELL

Telephone: (703) 308-9140 EXT 217



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
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Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING or 371(c) DATE	GRP ART UNIT	FIL FEE REC'D	ATTY. DOCKET NO	TOT CLAIMS	IND CLAIMS
11/587,893	05/22/2008	1661	3730	DAS-115XC1	67	4

CONFIRMATION NO. 7868

23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO BOX 142950
GAINESVILLE, FL 32614-2950

FILING RECEIPT



OC000000033724101

Date Mailed: 12/23/2008

Receipt is acknowledged of this non-provisional patent application. The application will be taken up for examination in due course. Applicant will be notified as to the results of the examination. Any correspondence concerning the application must include the following identification information: the U.S. APPLICATION NUMBER, FILING DATE, NAME OF APPLICANT, and TITLE OF INVENTION. Fees transmitted by check or draft are subject to collection. Please verify the accuracy of the data presented on this receipt. **If an error is noted on this Filing Receipt, please submit a written request for a Filing Receipt Correction. Please provide a copy of this Filing Receipt with the changes noted thereon. If you received a "Notice to File Missing Parts" for this application, please submit any corrections to this Filing Receipt with your reply to the Notice. When the USPTO processes the reply to the Notice, the USPTO will generate another Filing Receipt incorporating the requested corrections**

Applicant(s)

Terry R. Wright, Westfield, IN;
Justin M. Lira, Fishers, IN;
Donald J. Merlo, Carmel, IN;
Nicole L. Arnold, Carmel, IN;

Assignment For Published Patent Application

Dow AgroSciences LLC, Indianapolis, IN

Power of Attorney: The patent practitioners associated with Customer Number 23557

Domestic Priority data as claimed by applicant

This application is a 371 of PCT/US2005/014737 05/02/2005
which claims benefit of 60/567,052 04/30/2004

Foreign Applications

If Required, Foreign Filing License Granted: 12/19/2008

The country code and number of your priority application, to be used for filing abroad under the Paris Convention, is **US 11/587,893**

Projected Publication Date: 04/02/2009

Non-Publication Request: No

Early Publication Request: No

Title

Novel Herbicide Resistance Genes

Preliminary Class

PLT

PROTECTING YOUR INVENTION OUTSIDE THE UNITED STATES

Since the rights granted by a U.S. patent extend only throughout the territory of the United States and have no effect in a foreign country, an inventor who wishes patent protection in another country must apply for a patent in a specific country or in regional patent offices. Applicants may wish to consider the filing of an international application under the Patent Cooperation Treaty (PCT). An international (PCT) application generally has the same effect as a regular national patent application in each PCT-member country. The PCT process **simplifies** the filing of patent applications on the same invention in member countries, but **does not result** in a grant of "an international patent" and does not eliminate the need of applicants to file additional documents and fees in countries where patent protection is desired.

Almost every country has its own patent law, and a person desiring a patent in a particular country must make an application for patent in that country in accordance with its particular laws. Since the laws of many countries differ in various respects from the patent law of the United States, applicants are advised to seek guidance from specific foreign countries to ensure that patent rights are not lost prematurely.

Applicants also are advised that in the case of inventions made in the United States, the Director of the USPTO must issue a license before applicants can apply for a patent in a foreign country. The filing of a U.S. patent application serves as a request for a foreign filing license. The application's filing receipt contains further information and guidance as to the status of applicant's license for foreign filing.

Applicants may wish to consult the USPTO booklet, "General Information Concerning Patents" (specifically, the section entitled "Treaties and Foreign Patents") for more information on timeframes and deadlines for filing foreign patent applications. The guide is available either by contacting the USPTO Contact Center at 800-786-9199, or it can be viewed on the USPTO website at <http://www.uspto.gov/web/offices/pac/doc/general/index.html>.

For information on preventing theft of your intellectual property (patents, trademarks and copyrights), you may wish to consult the U.S. Government website, <http://www.stopfakes.gov>. Part of a Department of Commerce initiative, this website includes self-help "toolkits" giving innovators guidance on how to protect intellectual property in specific countries such as China, Korea and Mexico. For questions regarding patent enforcement issues, applicants may call the U.S. Government hotline at 1-866-999-HALT (1-866-999-4158).

LICENSE FOR FOREIGN FILING UNDER

Title 35, United States Code, Section 184

Title 37, Code of Federal Regulations, 5.11 & 5.15

GRANTED

The applicant has been granted a license under 35 U.S.C. 184, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" followed by a date appears on this form. Such licenses are issued in all applications where the conditions for issuance of a license have been met, regardless of whether or not a license may be required as

set forth in 37 CFR 5.15. The scope and limitations of this license are set forth in 37 CFR 5.15(a) unless an earlier license has been issued under 37 CFR 5.15(b). The license is subject to revocation upon written notification. The date indicated is the effective date of the license, unless an earlier license of similar scope has been granted under 37 CFR 5.13 or 5.14.

This license is to be retained by the licensee and may be used at any time on or after the effective date thereof unless it is revoked. This license is automatically transferred to any related applications(s) filed under 37 CFR 1.53(d). This license is not retroactive.

The grant of a license does not in any way lessen the responsibility of a licensee for the security of the subject matter as imposed by any Government contract or the provisions of existing laws relating to espionage and the national security or the export of technical data. Licensees should apprise themselves of current regulations especially with respect to certain countries, of other agencies, particularly the Office of Defense Trade Controls, Department of State (with respect to Arms, Munitions and Implements of War (22 CFR 121-128)); the Bureau of Industry and Security, Department of Commerce (15 CFR parts 730-774); the Office of Foreign Assets Control, Department of Treasury (31 CFR Parts 500+) and the Department of Energy.

NOT GRANTED

No license under 35 U.S.C. 184 has been granted at this time, if the phrase "IF REQUIRED, FOREIGN FILING LICENSE GRANTED" DOES NOT appear on this form. Applicant may still petition for a license under 37 CFR 5.12, if a license is desired before the expiration of 6 months from the filing date of the application. If 6 months has lapsed from the filing date of this application and the licensee has not received any indication of a secrecy order under 35 U.S.C. 181, the licensee may foreign file the application pursuant to 37 CFR 5.15(b).

UNITED STATES NATIONAL STAGE WORKSHEET (DO/EO)
B.C., Patent Application Specialist, PCT /DO/EO/National Stage Division

Publication No. WO 05 / 107437 Publication Date 11-17-05

Not Published: ☐ U.S. only ☐ Requested

Basic fee paid: \$ 300 PER fee paid: \$ * / US ISA fee paid: \$ * / US

U. S. Application No. 11/ 587,893

International Application Number: PCT/US2005 / 014737 International Filing Date: 05-02-05

Application Filed by 30 Month: ☒ Yes ☐ No Language: English

Copy of IA: yes ☒ no ☐ Translation: yes ☐ no ☐ Defective: ☐ Yes ☐ No Date satisfied: _____

371 Filing Fees ☒ paid ☐ Insufficient Meet Article 33 Requirement: ☒ Yes ☐ No & why not _____

Original claims: 1-68 Added Claims: _____ Total Claims: 67 Chargeable 67 Independent 4 multiple 4

After entry of Article 19 or 34 Amdt: _____

Cancelled Claims via Article 19 or 34 _____ &/or Pre-Amdt. _____ Clms. added via Article 19 or 34 _____

Total Number of Drawing Sheets: 24 Foreign Text: _____

Oath/Declaration: yes ☒ no ☐ signed ☒ unsigned ☐ defective: ☐ Date Satisfied: 5-22-08

PCT/RO/101/Request Form Declaration: ☐ yes properly executed ☐ No ☐ not properly executed Date: _____

Small Entity: ☐ Yes Small Entity Statement ☐ Assertion by filing fee paid ☐ Large Entity: ☒

1st Submission: Biochemical Seq. Diskette: yes ☒ no ☐ entered & date 11-8-06 not entered & date _____

2nd Submission: Biochemical Seq. Diskette: yes ☐ no ☐ entered & date _____ not entered & date _____

Biochemical Seq. Listing: yes ☒ no ☐ statement ☒ yes ☐ no other submission date(s): _____

Biochemical Diskette/Listing not needed: _____

Copy of ISR: ☐ with references ☐ without references ☒ Non-Establishment of ISR PCT/ISA/203 _____

Article 19 Amendment: ☐ entered ☐ not entered ☐ Replaced by Article 34 Amendment _____

Copy of IPER: ☐ without Annexes: ☐ with Annexes: ☐ Annexes entered ☐ Annexes not entered ☒ 237

Reason Annexes have not been entered: _____

Preliminary Amendment(s): yes ☒ not entered ☐ & why _____ Other Amendment dates: _____

IDS: ☒ yes ☒ with references ☐ without references Other IDS Dates: 3-24-08; 7-11-08

Request for Immediate Examination: yes ☒ no ☐ Other Early Processing Date: _____

Substitute Specification: yes ☐ Date filed: _____ Other documents filed: _____

Assignment: yes ☒ Date filed: 12-17-08 Assignment for PG Pub: ☐ Yes ☐ No Date filed: _____

Power of Attorney ☒ Date filed: 5/22/08 Application Data Sheet ☒ Priority Document(s): yes ☒

**Other: _____

**Other Problems: invention name change due to marriage office 71 opening

Application Size: Spec. 158 Claims 10 Abstract 1 Drawings 24 Seq. List. 23 Seq. RF 7 Total: (223)

Application Fees: owed (paid)

☒ Declaration ☒ Claims ☐ Multiple ☐ Translation ☐ Extension ☐ Petition ☐ Application size _____

Date of 35 USC Receipt of Request: 10-27-06

Date Completion USC 371 Requirements: 5/22/08 (decision 11-25-08)

Notice of Missing Requirements: 06/26/08 Response to Missing Requirements 8/21-08

371 Formalities Letter: (Sequence) 922 _____ 922 Response _____ or (Fees Owed) 923 _____ 923-Response _____

Notice of Defective Response: _____ Defective Response Reply _____

Notice of Acceptance: 12-19-08

Petition ☐ 8/21/08 Petition 1.47: _____ Petition Granted: 11-25-08

Notification of Abandonment: _____

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

POWER OF ATTORNEY OR REVOCATION OF POWER OF ATTORNEY WITH A NEW POWER OF ATTORNEY AND CHANGE OF CORRESPONDENCE ADDRESS	Application Number	11/587,893
	Filing Date	October 27, 2006
	First Named Inventor	Terry R. Wright
	Title	Novel Herbicide Resistant Gene
	Art Unit	1661
	Examiner Name	
	Attorney Docket Number	DAS-P0115-US-02

I hereby revoke all previous powers of attorney given in the above-identified application.

☐ A Power of Attorney is submitted herewith.

OR

☒ I hereby appoint Practitioner(s) associated with the following Customer Number as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

83067

OR

☐ I hereby appoint Practitioner(s) named below as my/our attorney(s) or agent(s) to prosecute the application identified above, and to transact all business in the United States Patent and Trademark Office connected therewith:

Practitioner(s) Name	Registration Number

Please recognize or change the correspondence address for the above-identified application to:

☒ The address associated with the above-mentioned Customer Number.

OR

☐ The address associated with Customer Number:

OR

☐ Firm or Individual Name

Address

City

State

Zip

Country

Telephone

Email

I am the:

☐ Applicant/Inventor.

OR

☒ Assignee of record of the entire interest. See 37 CFR 3.71.

Statement under 37 CFR 3.73(b) (Form PTO/SB/96) submitted herewith or filed on _____.

SIGNATURE of Applicant or Assignee of Record

Signature	<i>Andrea T. Borucki</i>	Date	1-15-09
Name	ANDREA T. BORUCKI	Telephone	317-337-4820
Title and Company	GENERAL PATENT COUNSEL, DOW AGROSCIENCES LLC		

NOTE: Signatures of all the inventors or assignees of record of the entire interest or their representative(s) are required. Submit multiple forms if more than one signature is required, see below*.

☒ *Total of 2 forms are submitted.

This collection of information is required by 37 CFR 1.31, 1.32 and 1.33. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 3 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

STATEMENT UNDER 37 CFR 3.73(b)Applicant/Patent Owner: Dow AgroSciences LLCApplication No./Patent No.: 11/587,893 Filed/Issue Date: October 27, 2006

Entitled:

Dow AgroSciences LLC

(Name of Assignee)

a Corporation

(Type of Assignee, e.g., corporation, partnership, university, government agency, etc.)

states that it is:

1. ☒ the assignee of the entire right, title, and interest; or
2. ☐ an assignee of less than the entire right, title and interest
(The extent (by percentage) of its ownership interest is _____ %)

in the patent application/patent identified above by virtue of either:

- A. ☒ An assignment from the inventor(s) of the patent application/patent identified above. The assignment was recorded in the United States Patent and Trademark Office at Reel 018985, Frame 0517, or for which a copy thereof is attached.

OR

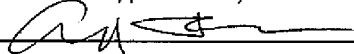
- B. ☐ A chain of title from the inventor(s), of the patent application/patent identified above, to the current assignee as follows:

1. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
2. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.
3. From: _____ To: _____
The document was recorded in the United States Patent and Trademark Office at Reel _____, Frame _____, or for which a copy thereof is attached.

☐ Additional documents in the chain of title are listed on a supplemental sheet.☐ As required by 37 CFR 3.73(b)(1)(i), the documentary evidence of the chain of title from the original owner to the assignee was, or concurrently is being, submitted for recordation pursuant to 37 CFR 3.11.

[NOTE: A separate copy (i.e., a true copy of the original assignment document(s)) must be submitted to Assignment Division in accordance with 37 CFR Part 3, to record the assignment in the records of the USPTO. See MPEP 302.08]

The undersigned (whose title is supplied below) is authorized to act on behalf of the assignee.



Signature

1-15-06

Date

Andrea T. Borucki(317) 337-4820

Printed or Typed Name

Telephone Number

General Patent Counsel

Title

This collection of information is required by 37 CFR 3.73(b). The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Electronic Acknowledgement Receipt	
EFS ID:	4684848
Application Number:	11587893
International Application Number:	
Confirmation Number:	7868
Title of Invention:	Novel Herbicide Resistance Genes
First Named Inventor/Applicant Name:	Terry R. Wright
Customer Number:	23557
Filer:	Jay M. Sanders/Lisa Schodrowski
Filer Authorized By:	Jay M. Sanders
Attorney Docket Number:	DAS-115XC1
Receipt Date:	27-JAN-2009
Filing Date:	22-MAY-2008
Time Stamp:	16:08:22
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no
------------------------	----

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1		DAS_P0115_POA.PDF	169883 1fe04695ebf6a3879a5a71d2ee3201dca71c e1c4	yes	2

	Multipart Description/PDF files in .zip description		
	Document Description	Start	End
	Power of Attorney	1	1
	CRF Statement Paper and CRF are the same	2	2
Warnings:			
Information:			
Total Files Size (in bytes):			169883
This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503. <u>New Applications Under 35 U.S.C. 111</u> If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application. <u>National Stage of an International Application under 35 U.S.C. 371</u> If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course. <u>New International Application Filed with the USPTO as a Receiving Office</u> If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.			



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
11/587,893	05/22/2008	Terry R. Wright	DAS-P0115-US-02

CONFIRMATION NO. 7868

POA ACCEPTANCE LETTER

83067
Baker & Daniels LLP- Dow AgroSciences
300 North Meridian Street, Suite 2700
Indianapolis, ID 46204



Date Mailed: 02/03/2009

NOTICE OF ACCEPTANCE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 01/27/2009.

The Power of Attorney in this application is accepted. Correspondence in this application will be mailed to the above address as provided by 37 CFR 1.33.

/lchau/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101



UNITED STATES PATENT AND TRADEMARK OFFICE

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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
11/587,893	05/22/2008	Terry R. Wright	DAS-115XC1

CONFIRMATION NO. 7868

POWER OF ATTORNEY NOTICE



23557
SALIWANCHIK LLOYD & SALIWANCHIK
A PROFESSIONAL ASSOCIATION
PO Box 142950
GAINESVILLE, FL 32614

Date Mailed: 02/03/2009

NOTICE REGARDING CHANGE OF POWER OF ATTORNEY

This is in response to the Power of Attorney filed 01/27/2009.

- The Power of Attorney to you in this application has been revoked by the assignee who has intervened as provided by 37 CFR 3.71. Future correspondence will be mailed to the new address of record(37 CFR 1.33).

/lchau/

Office of Data Management, Application Assistance Unit (571) 272-4000, or (571) 272-4200, or 1-888-786-0101

Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875					Application or Docket Number 11/587,893		Filing Date 05/22/2008		☐ To be Mailed	
---	--	--	--	--	---	--	----------------------------------	--	---------------------------------------	--

APPLICATION AS FILED – PART I						OTHER THAN SMALL ENTITY				
(Column 1)		(Column 2)		SMALL ENTITY ☐		OR		SMALL ENTITY		
FOR	NUMBER FILED	NUMBER EXTRA	RATE (\$)	FEE (\$)	OR	RATE (\$)	FEE (\$)	OR	RATE (\$)	FEE (\$)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A			N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*	X \$	=		X \$	=		X \$	=
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X \$	=		X \$	=		X \$	=
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$250 (\$125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).									
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))										
* If the difference in column 1 is less than zero, enter "0" in column 2.						TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II						OTHER THAN SMALL ENTITY					
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR		SMALL ENTITY	
AMENDMENT	10/27/2006	CLAIMS REMAINING AFTER AMENDMENT	MINUS	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	OR	RATE (\$)	ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))	* 67	Minus	** 67	=	X \$	=		X \$	=	
	Independent (37 CFR 1.16(h))	* 4	Minus	*** 4	=	X \$	=		X \$	=	
☐ Application Size Fee (37 CFR 1.16(s))											
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))											
						TOTAL ADD'L FEE			TOTAL ADD'L FEE		

(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR		SMALL ENTITY	
AMENDMENT	Total (37 CFR 1.16(i))	CLAIMS REMAINING AFTER AMENDMENT	MINUS	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	OR	RATE (\$)	ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$	=		X \$	=	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$	=		X \$	=	
☐ Application Size Fee (37 CFR 1.16(s))											
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))											
						TOTAL ADD'L FEE			TOTAL ADD'L FEE		

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
 ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
 *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
 The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1.

Legal Instrument Examiner:
/KATRINA S. TURNER/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.



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APPLICATION NUMBER	FILING OR 371(C) DATE	FIRST NAMED APPLICANT	ATTY. DOCKET NO./TITLE
11/587,893	05/22/2008	Terry R. Wright	DAS-P0115-US-02

CONFIRMATION NO. 7868

PUBLICATION NOTICE

83067
Baker & Daniels LLP- Dow AgroSciences
300 North Meridian Street, Suite 2700
Indianapolis, ID 46204



0000000035412010*

Title:Novel Herbicide Resistance Genes

Publication No.US-2009-0093366-A1

Publication Date:04/09/2009

NOTICE OF PUBLICATION OF APPLICATION

The above-identified application will be electronically published as a patent application publication pursuant to 37 CFR 1.211, et seq. The patent application publication number and publication date are set forth above.

The publication may be accessed through the USPTO's publicly available Searchable Databases via the Internet at www.uspto.gov. The direct link to access the publication is currently <http://www.uspto.gov/patft/>.

The publication process established by the Office does not provide for mailing a copy of the publication to applicant. A copy of the publication may be obtained from the Office upon payment of the appropriate fee set forth in 37 CFR 1.19(a)(1). Orders for copies of patent application publications are handled by the USPTO's Office of Public Records. The Office of Public Records can be reached by telephone at (703) 308-9726 or (800) 972-6382, by facsimile at (703) 305-8759, by mail addressed to the United States Patent and Trademark Office, Office of Public Records, Alexandria, VA 22313-1450 or via the Internet.

In addition, information on the status of the application, including the mailing date of Office actions and the dates of receipt of correspondence filed in the Office, may also be accessed via the Internet through the Patent Electronic Business Center at www.uspto.gov using the public side of the Patent Application Information and Retrieval (PAIR) system. The direct link to access this status information is currently <http://pair.uspto.gov/>. Prior to publication, such status information is confidential and may only be obtained by applicant using the private side of PAIR.

Further assistance in electronically accessing the publication, or about PAIR, is available by calling the Patent Electronic Business Center at 1-866-217-9197.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/587,893	05/22/2008	Terry R. Wright	DAS-P0115-US-02	7868
83067	7590	09/25/2009		
Baker & Daniels LLP- Dow AgroSciences 300 North Meridian Street, Suite 2700 Indianapolis, IN 46204			EXAMINER KRUSE, DAVID H	
			ART UNIT	PAPER NUMBER
			1638	
			NOTIFICATION DATE	DELIVERY MODE
			09/25/2009	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

intcas@bakerd.com
cynthia.payson@bakerd.com

Office Action Summary	Application No. 11/587,893	Applicant(s) WRIGHT ET AL.	
	Examiner David H. Kruse	Art Unit 1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 1 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☐ Responsive to communication(s) filed on ____.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) 1-51 and 53-68 is/are pending in the application.

4a) Of the above claim(s) ____ is/are withdrawn from consideration.

5) ☐ Claim(s) ____ is/are allowed.

6) ☐ Claim(s) ____ is/are rejected.

7) ☐ Claim(s) ____ is/are objected to.

8) ☒ Claim(s) 1-51 and 53-68 are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☐ The drawing(s) filed on ____ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) ☐ All b) ☐ Some * c) ☐ None of:
 1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO/SB/08)
 Paper No(s)/Mail Date ____.

4) ☐ Interview Summary (PTO-413)
 Paper No(s)/Mail Date ____.

5) ☐ Notice of Informal Patent Application

6) ☐ Other: ____.

DETAILED ACTION

Election/Restrictions

1. Restriction is required under 35 U.S.C. 121 and 372.

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1.

In accordance with 37 CFR 1.499, applicant is required, in reply to this action, to elect a single invention to which the claims must be restricted.

Group I, claim(s) 1-51 and 57-68, drawn to a transgenic plant cell comprising a polynucleotide that encodes and AAD-1 protein and methods of using and making.

Group II, claim(s) 53-56, drawn to a method of detecting whether a plant comprises a polynucleotide that encodes and AAD-1 protein.

2. The inventions listed as Groups I and II do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The invention of Group II is directed to a method of using an antibody or PCR primer which is a distinct inventive concept from the invention of Group I directed to use of a polynucleotide that encodes and AAD-1 protein, hence the groups lack the same special technical feature.

3. Applicant is advised that the reply to this requirement to be complete within one month (not less than 30 days) must include an election of the invention to be examined even though the requirement be traversed (37 CFR 1.143).

4. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR § 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR § 1.48(b) and by the fee required under 37 CFR § 1.17(i).

5. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David H. Kruse, Ph.D. whose telephone number is (571)

Application/Control Number: 11/587,893

Page 3

Art Unit: 1638

272-0799. The examiner can normally be reached on Monday to Friday from 8:00 a.m. to 4:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg can be reached at (571) 272-0975. The fax telephone number for this Group is (571) 273-8300.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group Receptionist whose telephone number is (571) 272-1600.

/David H. Kruse/
Primary Examiner, Art Unit 1638
21 September 2009

To: inteias@bakerd.com,cynthia.payson@bakerd.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 83067

Sep 25, 2009 05:42:48 AM

Dear PAIR Customer:

Baker & Daniels LLP- Dow AgroSciences
300 North Meridian Street, Suite 2700
Indianapolis, IN 46204
UNITED STATES

The following USPTO patent application(s) associated with your Customer Number, 83067 , have new outgoing correspondence. This correspondence is now available for viewing in Private PAIR.

The official date of notification of the outgoing correspondence will be indicated on the form PTOL-90 accompanying the correspondence.

Disclaimer:

The list of documents shown below is provided as a courtesy and is not part of the official file wrapper. The content of the images shown in PAIR is the official record.

Application	Document	Mailroom Date	Attorney Docket No.
11587893	CTRS	09/25/2009	DAS-P0115-US-02

To view your correspondence online or update your email addresses, please visit us anytime at <https://sportal.uspto.gov/secure/myportal/privatepair>.

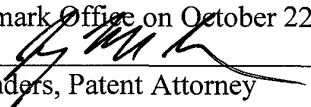
If you have any questions, please email the Electronic Business Center (EBC) at EBC@uspto.gov with 'e-Office Action' on the subject line or call 1-866-217-9197 during the following hours:

Monday - Friday 6:00 a.m. to 12:00 a.m.

Thank you for prompt attention to this notice,

UNITED STATES PATENT AND TRADEMARK OFFICE
PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM

I hereby certify that this correspondence if being electronically filed with the United States Patent and Trademark Office on October 22, 2009


Jay M. Sanders, Patent Attorney

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

<i>Art Unit:</i>	1638	)	Confirmation No. 7868
		)	
<i>Attorney</i>		)	
<i>Docket No.:</i>	DAS-P0115-US-02	)	
		)	
<i>Inventors:</i>	Terry Wright et al.	)	
		)	
<i>Title:</i>	Novel Herbicide Resistance Genes	)	
		)	
<i>Serial No:</i>	11/587,893	)	
		)	
<i>Filed:</i>	May 22, 2008	)	
		)	
<i>Examiner:</i>	David H. Kruse	)	

ELECTION/RESPONSE TO RESTRICTION REQUIREMENT

In response to the Restriction Requirement mailed September 25, 2009, Applicants hereby elect Group I (Claims 1-51 and 57-68).

Applicants believe that no fees are due in connection with this submission. However, if any fees are necessary, please charge Deposit Account No. 020390.

Respectfully submitted,

By:


Jay M. Sanders
Reg. No. 39,355
BAKER & DANIELS
300 N. Meridian Street, Suite 2700
Indianapolis, Indiana 46204
Telephone: (317) 237-1245
Fax: (317) 237-1000

Electronic Acknowledgement Receipt	
EFS ID:	6309942
Application Number:	11587893
International Application Number:	
Confirmation Number:	7868
Title of Invention:	Novel Herbicide Resistance Genes
First Named Inventor/Applicant Name:	Terry R. Wright
Customer Number:	83067
Filer:	Jay M. Sanders
Filer Authorized By:	
Attorney Docket Number:	DAS-P0115-US-02
Receipt Date:	22-OCT-2009
Filing Date:	22-MAY-2008
Time Stamp:	15:42:26
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	no
------------------------	----

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Response to Election / Restriction Filed	DAS_P115_Response_Restriction.PDF	41875 084de0b99d418d756f8e101062071ee63a3e480	no	1

Warnings:

Information:

This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.

New Applications Under 35 U.S.C. 111

If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.

National Stage of an International Application under 35 U.S.C. 371

If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.

New International Application Filed with the USPTO as a Receiving Office

If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.



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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/587,893	05/22/2008	Terry R. Wright	DAS-P0115-US-02	7868
83067	7590	03/09/2010		
Baker & Daniels LLP- Dow AgroSciences 300 North Meridian Street, Suite 2700 Indianapolis, IN 46204			EXAMINER KRUSE, DAVID H	
			ART UNIT	PAPER NUMBER
			1638	
			NOTIFICATION DATE	DELIVERY MODE
			03/09/2010	ELECTRONIC

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

intcas@bakerd.com
cynthia.payson@bakerd.com

Office Action Summary	Application No. 11/587,893	Applicant(s) WRIGHT ET AL.	
	Examiner David H. Kruse	Art Unit 1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1) ☒ Responsive to communication(s) filed on 22 October 2009.

2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.

3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4) ☒ Claim(s) 1-51 and 53-68 is/are pending in the application.

4a) Of the above claim(s) 53-56 is/are withdrawn from consideration.

5) ☐ Claim(s) _____ is/are allowed.

6) ☒ Claim(s) 1-51 and 57-68 is/are rejected.

7) ☐ Claim(s) _____ is/are objected to.

8) ☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9) ☐ The specification is objected to by the Examiner.

10) ☒ The drawing(s) filed on 27 October 2006 is/are: a) ☒ accepted or b) ☐ objected to by the Examiner.
 Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).
 Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12) ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 a) ☐ All b) ☐ Some * c) ☐ None of:
 1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. _____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☒ Information Disclosure Statement(s) (PTO/SB/08)
 Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413)
 Paper No(s)/Mail Date. _____.

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

Election/Restrictions

1. Applicant's election of Group I, claims 1-51 and 57-68 in the reply filed on 22 October 2009 is acknowledged. Because applicant did not distinctly and specifically point out the supposed errors in the restriction requirement, the election has been treated as an election without traverse (MPEP § 818.03(a)).
2. Claims 53-56 are withdrawn from further consideration pursuant to 37 CFR 1.142(b) as being drawn to a nonelected invention, there being no allowable generic or linking claim. Election was made **without** traverse in the reply filed on 22 October 2009.
3. Applicant is reminded that upon the cancellation of claims to a non-elected invention, the inventorship must be amended in compliance with 37 CFR 1.48(b) if one or more of the currently named inventors is no longer an inventor of at least one claim remaining in the application. Any amendment of inventorship must be accompanied by a request under 37 CFR 1.48(b) and by the fee required under 37 CFR 1.17(i).

Claim Objections

4. Claim 1 is objected to because of the following informalities: At line 1, -- recombinant -- should be inserted before "polynucleotide". Appropriate correction is required.

Claim 40 is objected to because of the following informalities: At line 3, "a cell" should read -- the cell -- in referring to claim 1. Appropriate correction is required.

Claim 50 is objected to because of the following informalities: At line 2, 'a polynucleotide" should read -- the polynucleotide -- in referring to claim 47. Appropriate correction is required. See also claim 64, line 2.

Claim 57 is objected to because of the following informalities: "a cell" should read -- the cell -- in referring to claim 1. Appropriate correction is required.

Claim Rejections - 35 USC § 112

5. The following is a quotation of the second paragraph of 35 U.S.C. 112:

The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

6. Claim 60 is rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The recitation of "method is used" renders the claim indefinite because the metes and bounds of the claim are unclear. This is because the method of claim 13 is to "controlling at least one weed in a field", one is applying an herbicide and it is unclear to what herbicide the weeds would be resistant to. If the weeds are resistant to a phenoxy auxin herbicide or an aryloxyphenoxypropionate herbicide then there would be no control of weeds, if the weeds are not so resistant then it is unclear what the instant claim encompasses.

7. The following is a quotation of the first paragraph of 35 U.S.C. 112:

The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

8. Claims 44-51 and 63-68 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention.

Applicants claim a polynucleotide optimized for expression in a plant encoding a protein having aryloxyalkanoate dioxygenase activity that hybridizes under stringent conditions with the full complement of a sequence selected from SEQ ID NO: 3, 4 or 5 and a method of using same. Applicants claim a method of conferring 2,4-D herbicide resistance to a crop by introducing a nucleic acid encoding an enzyme having R-specific aryloxyalkanoate dioxygenase activity.

Applicant describes polynucleotides encoding an enzyme having aryloxyalkanoate dioxygenase activity at SEQ ID NO: 9, 10 and 11 which are more than 99% identical to each other.

Applicants do not describe other enzymes having aryloxyalkanoate dioxygenase activity, nor does Applicants describe any specific structure of a dioxygenase that produces aryloxyalkanoate dioxygenase activity.

Hence, it is unclear that Applicants were in possession of the invention as broadly claimed. See *University of California V. Eli Lilly and Co.*, 43 USPQ2d 1398 (Fed. Cir. 1997), which teaches that the disclosure of a process for obtaining cDNA from a particular organism and the description of the encoded protein fail to provide an adequate written description of the actual cDNA from that organism which would

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encode the protein from that organism, despite the disclosure of a cDNA encoding that protein from another organism. At 1406, the court states that a description of a genus of cDNAs may be achieved by means of a recitation of a representative number of cDNAs, defined by nucleotide sequence, falling within the scope of the genus or of a recitation of structural features common to the members of the genus, which features constitute a substantial portion of the genus. A method is not described if products used in the method are not described. See 64 Fed. Reg. 71427, 71428 (1999), comment No. 4.

Claim Rejections - 35 USC § 103

9. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

10. Claim 59 is rejected under 35 U.S.C. 103(a) as being unpatentable over Spencer *et al* (1992 Plant Molecular Biology 18: 201-210).

Applicants claim progeny of a transgenic plant.

Spencer teaches that transgenes will segregate in a Mendelian (1:1) fashion in progeny of a transgenic plant.

Spencer does not teach the transgenic plant of instant claim 5, upon which claim 59 depends.

The instant claim is *prima face* obvious over the teaching of Spencer *et al* because the claimed progeny would comprise individual that lack a transgene, said individual would be obvious in view of the teachings of Spencer *et al*.

11. Claims 1-51 and 57-68 are rejected under 35 U.S.C. 103(a) as being unpatentable over Kaphammer (U.S. Patent 5,608,147) in view of Kohler (1999 J. Industrial Microbiology & Biotechnology 23: 336-340) and van der Meer (3 March 2004, Genbank Accession No. AJ628859, NCBI-National Library of Medicine USA, Bethesda, Maryland) and in further view of Jepson *et al* (U.S. Patent Application Publication US 2003/0041357).

Kaphammer teaches transgenic plant cells, plants and seeds transformed with a *tfdA* (2,4-dichlorophenoxyacetate (2,4-D)/ α -ketoglutarate dioxygenase) gene that confers tolerance to the herbicide 2,4-D (claims 11-23).

Kaphammer does not teach a polynucleotide that encodes an aryloxyalkanoate dioxygenase that is at least 95% identical to instant SEQ ID NO: 9. Kaphammer does not teach introducing a transgene encoding an enzyme that produces tolerance to a different herbicide.

van der Meer teaches a polynucleotide isolated from *Sphingobium herbicidovorans* MH that encodes an enzyme that degrades (R)-dichlorprop and (R)-mecoprop said enzyme being 100% identical to instant SEQ ID NO: 9. Kohler teaches that *Sphingobium herbicidovorans* MH degrades achiral phenoxyacetic acid herbicides (see Table 1, page 338), in particular 2,4-D, (R)-dichlorprop and (R)-mecoprop.

Jepson *et al* teaches that at the time of Applicants' invention, it would have been obvious to one of ordinary skill in the art to introduce additional transgenes to produce transgenic plant having resistances to multiple herbicides (see the Abstract) including the product of a *tfdA* gene (see claim 4).

It would have been *prima face* obvious to one of ordinary skill in the art at the time of Applicants' invention to modify the teachings of Kaphammer using the polynucleotide taught by van der Meer to produce a transgenic plant cell having resistance to an aryloxyphenoxypropionate herbicide. It would also have been obvious to modify the teachings of Kaphammer using the teachings of Jepson *et al* to introduce an additional polynucleotide encoding a second herbicide resistance in conjunction with a *tfdA* type gene. Given the teachings of Kohler, one of ordinary skill in the art at the time of Applicants' invention would have had a reasonable expectation of success to produce a transgenic plant resistance to an aryloxyphenoxypropionate herbicide. Given the teachings of Jepson *et al*, one of ordinary skill in the art would have had a reasonable expectation of success in adding a second herbicide resistance gene.

Double Patenting

12. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory obviousness-type double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422

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F.2d 438, 164 USPQ 619 (CCPA 1970); and *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on a nonstatutory double patenting ground provided the conflicting application or patent either is shown to be commonly owned with this application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement.

Effective January 1, 1994, a registered attorney or agent of record may sign a terminal disclaimer. A terminal disclaimer signed by the assignee must fully comply with 37 CFR 3.73(b).

13. Claims 1-51 and 57-68 are provisionally rejected on the ground of nonstatutory obviousness-type double patenting as being unpatentable over claims 99-126 of copending Application No. 12/599,385. Although the conflicting claims are not identical, they are not patentably distinct from each other because the instant claims overlap in scope with the claims of the copending application.

This is a provisional obviousness-type double patenting rejection because the conflicting claims have not in fact been patented.

Conclusion

14. No claims are allowed.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to David H. Kruse, Ph.D. whose telephone number is (571) 272-0799. The examiner can normally be reached on Monday to Friday from 8:00 a.m. to 4:30 p.m.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anne Marie Grunberg can be reached at (571) 272-0975. The central FAX number for official correspondence is 571-273-8300.

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Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the Group Receptionist whose telephone number is (571) 272-1600.

/David H Kruse/
Primary Examiner, Art Unit 1638
26 February 2010

Notice of References Cited	Application/Control No. 11/587,893		Applicant(s)/Patent Under Reexamination WRIGHT ET AL.	
	Examiner David H. Kruse		Art Unit 1638	Page 1 of 1

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,608,147	03-1997	Kaphammer, Bryan J.	800/294
*	B	US-2003/0041357	02-2003	Jepson et al.	800/300
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Spencer et al 1992 Plant Molecular Biology 18: 201-210.
*	V	Kohler 1999 J. Industrial Microbiology & Biotechnology 23: 336-340
	W	van der Meer, 3 March 2004, Genbank Accession No. AJ628859, NCBI-National Library of Medicine USA, Bethesda, Maryland
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

Search Notes 	Application/Control No. 11587893	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.
	Examiner David H Kruse	Art Unit 1638

SEARCHED			
Class	Subclass	Date	Examiner
EAST	EAST	2/24/2010	/DK/

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor Name Search	2/25/2010	/DK/
EAST: USPAT, USPGPUB, EPO, JPO, DERWENT	2/25/2010	/DK/
STN: AGRICOLA, BIOSIS, CAPLUS, EMBASE	2/26/2010	/DK/
Sequence Search SEQ ID NO: 9, included interference search.	12/17/2009	/DK/

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner

	/David H Kruse/ Primary Examiner.Art Unit 1638
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)				Complete if Known	
				Application Number	11/587,893
				Filing Date	October 27, 2006
				First Named Inventor	Terry Wright
				Art Unit	1661
				Examiner Name	
Sheet	1	of	2	Attorney Docket Number	DAS-115XC1

U.S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number - Kind Code ² (if known)			
/DK/	U1	US-7,250,561	07-31-2007	Pallett <i>et al.</i>	ALL
	U2	US-			
	U3	US-			
	U4	US-			
	U5	US-			
	U6	US-			
	U7	US-			

FOREIGN PATENT DOCUMENTS						
Examiner Initials*	Cite No. ¹	Foreign Patent Document	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T ⁶
		Country Code ³ - Number ⁴ - Kind Code ⁵ (if known)				
/DK/	F1	WO 98/02562	01-22-1998	Rhone-Poulenc Agrochimie	ALL	
/DK/	F2	WO 03/034813	05-01-2003	The United States of America, as Represented by the Secretary of Agriculture	ALL	
	F3					
	F4					
	F5					
	F6					
	F7					

Examiner Signature	/David Kruse/ (02/25/2010)	Date Considered	
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Substitute for form 1449A/PTO			Complete if Known		
INFORMATION DISCLOSURE STATEMENT BY APPLICANT (use as many sheets as necessary)			Application Number	11/587,893	
			Filing Date	October 27, 2006	
			First Named Inventor	Terry Wright	
			Art Unit	1661	
			Examiner Name		
Sheet	2	of	2	Attorney Docket Number	DAS-115XC1

NON PATENT LITERATURE DOCUMENTS				
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article, (when appropriate), title of the magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.		
/DK/	R1	Database EMBL, Accession No. AJ628859, KOHLER, H.P.E. "Sphingomonas herbicidovorans tnpA gene for transposase and rdpA gene for (R)-2-(2,4-dichlorophenoxy)propionate,2-oxoglutarate dioxygenase", March 3, 2004, ~~XP-692474825~~ , pp. 1-2.		
	R2	KOHLER, H.P.E. "Sphingomonas herbicidovorans MH: A versatile phenoxyalkanoic acid herbicide degrader" <i>Journal of Industrial Microbiology & Biotechnology</i> , October 1999, pp. 336-340, Vol. 23, No. 4-5		
	R3	Database EMBL, Accession No. AF516751, SCHLEINITZ, K.M. <i>et al.</i> , "Rhodoferrax sp. P230 R-2,4-dichlorophenoxypropionate dioxygenase (rdpA) gene, complete cds.", August 6, 2002, ~~XP-692474826~~ , pp. 1-2.		
	R4	Database EMBL, Accession No. AF516752, SCHLEINITZ, K.M. <i>et al.</i> , "Sphingobium herbicidovorans R-2,4-dichlorophenoxypropionate dioxygenase (rdpA) gene, complete cds.", August 6, 2002, ~~XP-692474827~~ , pp. 1-2.		
	R5	SCHLEINITZ, K.M. "Localization and Characterization of Two Novel Genes Encoding Stereospecific Dioxygenases Catalyzing 2(2,4-Dichlorophenoxy)propionate cleavage in <i>Delftia acidovorans</i> MC1" <i>Applied and Environmental Microbiology</i> , September 2004, pp. 5357-5365, Vol. 70, No. 9.		
/DK/	R6	Supplemental European Search Report dated March 28, 2008		

Examiner Signature	/David Kruse/ (02/25/2010)	Date Considered	
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EAST Search History**EAST Search History (Prior Art)**

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	5	plant and tfda.clm.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2010/02/25 17:23
L2	28	aryloxyalkanoate adj dioxygenase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2010/02/25 17:31

2/ 25/ 10 5:35:01 PM**C:\ Documents and Settings\ dkruse\ My Documents\ EAST\ Workspaces\ 11587893.wsp**

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Substitute for form 1449/PTO INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>		Complete if Known			
		Application Number	11/587,893		
		Filing Date	October 27, 2006		
		First Named Inventor	Terry Wright		
		Art Unit	Not Yet Known		
		Examiner Name	Not Yet Known		
Sheet	1	of	2	Attorney Docket Number	DAS-115XC1

U. S. PATENT DOCUMENTS					
Examiner Initials*	Cite No. ¹	Document Number	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
		Number-Kind Code ² (if known)			
/DK/	1	US- 5,273,894	12/28/1993	Strauch et al.	(Please review all)
	2	US- 5,561,236	10/01/1996	Leemans et al.	(Please review all)
	3	US- 5,608,147	03/04/1997	Kaphammer	(Please review all)
	4	US- 5,637,489	06/10/1997	Strauch et al.	(Please review all)
	5	US- 5,646,024	07/08/1997	Leemans et al.	(Please review all)
	6	US- 5,648,477	07/15/1997	Leemans et al.	(Please review all)
	7	US- 5,879,903	03/09/1999	Strauch et al.	(Please review all)
	8	US- 6,153,401	11/28/2000	Streber et al.	(Please review all)
/DK/	9	US- 7,112,665	09/26/2001	Leemans et al.	(Please review all)
		US-			
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FOREIGN PATENT DOCUMENTS						
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		Country Code ³ -Number ⁴ -Kind Code ⁵ (if known)				

Examiner Signature	/David Kruse/ (02/25/2010)	Date Considered	
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INFORMATION DISCLOSURE STATEMENT BY APPLICANT <i>(Use as many sheets as necessary)</i>		Complete if Known	
		Application Number	11/587,893
		Filing Date	October 27, 2006
		First Named Inventor	Terry Wright
		Art Unit	Not Yet Known
		Examiner Name	Not Yet Known
Sheet 2 of 2	Attorney Docket Number	DAS-115XC1	

NON PATENT LITERATURE DOCUMENTS			
Examiner Initials*	Cite No. ¹	Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.	T ²
/DK/	10	GENBANK Accession No. M16730. April 1993	
	11	HORVATH, M. et al., "Isolation and characterization of a 2-(2,4-dichlorophenoxy)propionic acid-degrading...", Appl. Microbiol Biotechnol., 1990, pgs.33;213-216. (Abstract only)	
	12	KOHLER, H.P.E., Sphingobium herbicidovorans MH: a versatile phenoxyalkanoic acid...; J. Ind Microbiol and Biotech; 1999; pgs 23:336-340. (Abstract only)	
	13	LYON, B.R. et al., "Expression of a bacterial gene in transgenic tobacco confers resistance to the herbicide 2,4-dichlorophenoxyacetic acid" Transgenic Res. 1989, pgs.33:166-169.	
	14	LYON, B.R. et al., "Cotton plants transformed with a bacterial degradation gene are protected from accidental..."; Transgenic Res.; 1993; pgs2:166-169. (Abstract only)	
	15	SMEJKAL, C.W. et al., "Substrate specificity of chlorophenoxyalkanoic acid-degrading bacteria..."; Biol. Fertil., 2001; pgs 33:507-513.	
	16	STREBER, W. et al., "Transgenic tobacco plants expressing a bacterial detoxifying enzyme are resistant to 2,4-D"; Bio/Technology; 1989; pgs 7:811-816. (Abstract only)	
	17	STREBER, W.R. et al., "Analysis, cloning, and high-level expression of 2,4-dichlorophenylacetic monooxygenase gene..."; J. Bacteriol; 1987; pgs 169:2950-2955.	
	18	WESTENDORF, A. et al., "The two enantiospecific dichlorprop/alpha-ketoglutarate-dioxygenases..."; Microbiol. Res.; 2002; pgs 157:317-22. (Abstract only)	
/DK/	19	WESTENDORF, A., "Purification and characterization of the enantiospecific dioxygenases from Delftia acidovorans..."; Acta Biotechnol.; 2003; pgs23:3-17.	

Examiner Signature	/David Kruse/ (02/25/2010)	Date Considered	
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¹ Applicant's unique citation designation number (optional). ² Applicant is to place a check mark here if English language Translation is attached.
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LOCUS AJ628859 2693 bp DNA linear BCT 03-MAR-2004
DEFINITION Sphingomonas herbicidovorans tnpA gene for transposase and rdpA
gene for (R)-2-(2,4-dichlorophenoxy)propionate,2-oxoglutarate
dioxxygenase.
ACCESSION AJ628859
VERSION AJ628859.1 GI:44903448
KEYWORDS (R)-2-(2,4-dichlorophenoxy)propionate,2-oxoglutarate dioxxygenase;
rdpA gene; tnpA gene; transposase.
SOURCE Sphingobium herbicidovorans (Sphingomonas herbicidovorans)
ORGANISM Sphingobium herbicidovorans
Bacteria; Proteobacteria; Alphaproteobacteria; Sphingomonadales;
Sphingomonadaceae; Sphingobium.
REFERENCE 1
AUTHORS Kohler,H.P.
TITLE Sphingomonas herbicidovorans MH: a versatile phenoxyalkanoic acid
herbicide degrader
JOURNAL J. Ind. Microbiol. Biotechnol. 23 (4-5), 336-340 (1999)
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Biologie, Lausanne, CH 1015, SWITZERLAND
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Segregation of transgenes in maize

T. Michael Spencer, James V. O'Brien, William G. Start, Thomas R. Adams,
William J. Gordon-Kamm, Peggy G. Lemaux¹

Discovery Research, DEKALB Plant Genetics, Eastern Point Road, Groton, CT 06340, USA; ¹*present address: Department of Plant Biology, University of California, Berkeley, CA 94720, USA*

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Abstract

Progeny recovered from backcrossed transgenic maize tissue culture regenerants (R_0) were analyzed to determine the segregation, expression, and stability of the introduced genes. Transgenic A188 $\times$ B73 R_0 plants (regenerated from embryogenic suspension culture cells transformed by microprojectile bombardment; see [9]) were pollinated with nontransformed B73 pollen. Inheritance of a selectable marker gene, *bar*, and a nonselectable marker gene, *uidA*, was analyzed in progeny (R_1) representing four independent transformation events. Activity of the *bar* gene product, phosphinothricin acetyltransferase (PAT), was assessed in plants comprising the four R_1 populations. The number of R_1 plants containing PAT activity per total number of R_1 plants recovered for each population was 2/7, 19/34, 3/14 and 73/73. Molecular analysis confirmed the segregation of *bar* in three R_1 populations and the lack of segregation in one R_1 population. Cosegregation analysis indicated genetic linkage of *bar* and *uidA* in all four R_1 populations. Analysis of numerous R_2 plants derived from crossing transformed R_1 plants with non-transformed inbreds revealed 1:1 segregation of PAT activity in three of four lines, including the line that failed to segregate in the R_1 generation. Integrated copies of *bar* in one line appeared to be unstable or poorly transmitted.

Introduction

Major progress has been made in overcoming the recalcitrance of agronomically important monocots to genetic transformation. Rice yielded to transformation via protoplast-based, direct DNA uptake systems, ultimately giving rise to fertile transgenic japonica [17] and indica [4] rice plants. Recovery of fertile transgenic maize plants [9, 8] was recently accomplished using microprojectile bombardment, a direct DNA delivery system not requiring protoplast isolation [13]. While

this progress is encouraging, very little is known about the integration and expression, and ultimately, the inheritance and stability of genes introduced into important monocots.

Fertile transgenic plants have been recovered from numerous dicot species using *Agrobacterium*-mediated transformation. *Agrobacterium* transformation of dicots usually results in a low number (average of 3 copies) of T-DNA insertions into the host plant genome. T-DNA insertions appear to be site-independent and are often unlinked (for review, see [22]). Inheritance

of introduced genes is usually Mendelian [7, 1, 5, 11]. Genes introduced into dicots via *Agrobacterium* appear to be very stable; however, low frequencies of instability have been observed [16, 3].

Inheritance of genes introduced via direct DNA delivery systems, particularly microprojectile bombardment, has not been as well characterized. Genes on gold particles accelerated into soybean meristems resulted in chimeric regenerants containing expressing and nonexpressing sectors; germ-line transformation was observed at low frequencies [15]. Analysis of subsequent generations of two lines of transgenic soybean showed that all copies of foreign genes were inherited in Mendelian fashion as single units [2]. Transgenic tobacco lines recovered from bombarded leaf discs exhibited both Mendelian and non-Mendelian segregation for introduced genes [21]. Here, we describe detailed analysis of inheritance, expression, and stability of genes introduced into cultured embryogenic maize cells by microprojectile bombardment.

Materials and methods

Plant care and backcrossing

Tissue culture regenerants (R_0) were grown to maturity in a greenhouse in 16 liter pots containing modified Promix BX (Premier Brands, Stamford, CT). Plants received a 14 h photoperiod of natural and supplemental light (high-pressure sodium lamps, $250 \mu\text{E m}^{-2} \text{s}^{-1}$). R_0 ears were pollinated with pollen from nontransformed, seed-derived B73 plants. Kernels that developed on R_0 ears were removed 30–40 days after pollination and planted in 0.5 liter pots. R_1 plants were grown to the 1–2 leaf stage in a growth chamber ($450 \mu\text{E m}^{-2} \text{s}^{-1}$; 14 h photoperiod) and subsequently transferred to 16 liter pots and grown to maturity in the greenhouse. Progeny (R_1) were crossed, as both the male and female parent, to several nontransformed inbred lines. Kernels recovered as a result of R_1 crosses matured on the plants for 40–45 days, were removed from the ears, and handled as described above.

Enzyme activity determination

Phosphinothricin acetyltransferase (PAT) activity in R_1 plants was assessed by local application of a 2% (v/v) Basta (Hoechst, FRG) solution to leaves as described [9]. The Basta formulation used, Basta TX, contains 200 g/l glufosinate (ammonium salt of phosphinothricin). PAT activity in R_2 plants was assessed by spraying plants at approximately the three-leaf stage with a 1% (v/v) Basta solution (manufacturer's recommended application rate is 0.5–1%) containing 0.1% (v/v) Triton. Herbicide was applied as a fine mist using a Spra-tool aerosol dispenser (Crown Industrial Products Co., Hebron, IL) until all leaf surfaces were wet. Plants were evaluated for resistance to the herbicide one week after spraying.

Southern blot analysis

Genomic DNA isolation from leaf tissue and Southern blot analyses were performed as described [9]. Approximately 5 μg genomic DNA was used per digest. The 0.6 kb *Sma* I fragment from pDPG165 [9] containing *bar* and the 1.8 kb *Bam* HI/*Eco* RI fragment from pCEV5 [18] containing *uidA* were used in random priming reactions ([6], Boehringer Mannheim) to generate probes for detecting PAT- and GUS-coding sequences, respectively.

Results and discussion

In a previous report [9] we described regeneration of 219 plants (R_0) from transformed callus lines recovered from transformation experiments utilizing an embryogenic A188 $\times$ B73 maize suspension culture designated SC716. In these experiments, SC716 suspension culture cells were bombarded with tungsten particles coated with two plasmids, one containing *bar* (pDPG165) and the other containing *uidA* (pDPG208). The plasmids pDPG165 (35S-*bar*-Tr7 3' end) and pDPG208 (35S-*adh1* intron-GUS-*nos* 3' end)

have been described [9]. Transformed callus lines were selected using the herbicide bialaphos. Transformed SC716 callus lines, previously designated R1-R21 [9], will here be referred to as T1-T21 to avoid confusion with designations for plant generations (e.g. R_0 , R_1 , and R_2). Plants were regenerated from nine independent SC716 transformants. Two of the ten independent SC716 transformants described by Gordon-Kamm *et al.* [9] were found to be identical upon further Southern analysis (T6, T11; data not shown).

Analysis of R_0 plants

The hybridization patterns of *bar* sequences in SC716 R_0 plants and callus were analyzed to determine if the sequences were stably maintained during plant regeneration and to confirm the clonal origin of the individual callus lines. Southern blot analyses of genomic DNA from each of the nine regenerable callus lines, and two R_0 plants derived from each callus line, are shown in Fig. 1; an additional 18 plants (2–6 plants/callus line) were analyzed for a total of 36 plants (data

not shown). Genomic DNA was digested with *Eco* RI and *Hind* III, which release the 1.9 kb *bar* expression unit (35S promoter-*bar*-Tr7 3' end) from pDPG165 [9]. Hybridization patterns for all 36 R_0 plants were identical to their corresponding callus lines with the exception of one of the two T5 R_0 plants analyzed (Fig. 1). It is possible that callus line T5 arose from more than one transformed cell and the dissimilar hybridization patterns of the two R_0 plants represent independent transformation events. Or, if callus line T5 originated from a single-cell transformation event, the T5 R_0 plant that exhibited a different hybridization pattern may have resulted from cells that lost some copies of the integrated plasmid DNA.

While only 36 of 219 R_0 plants were analyzed for the presence of *bar*, these 36 plants were randomly selected and all but one exhibited *bar* hybridization profiles identical to that of corresponding callus. These comparisons of hybridization patterns from callus and R_0 plants, as well as similar analyses of four independent transformation events described previously [9], provide strong evidence for lack of genetic chimerism in bialaphos-selected callus lines.

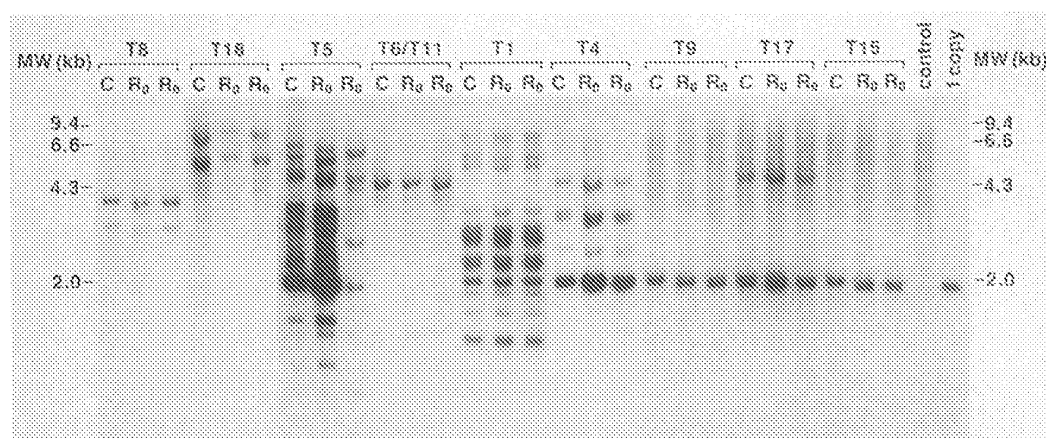


Fig. 1. Southern blot analyses of *bar* in callus (C) and corresponding primary regenerants (R_0). Genomic DNA from the nine regenerable callus lines (T8, T18, T5, T6/T11, T1, T4, T9, T17, and T15) and two plants regenerated from each callus line was digested with *Eco* RI and *Hind* III and probed with *bar*. Control DNA was from a nontransformed A188 × B73 tissue culture regenerant. Lane designated 1 copy contained 2.3 pg of the 1.9 kb *Eco* RI/*Hind* III *bar* expression unit from pDPG165, representing one copy per diploid genome. Molecular weights (MW) of standards are shown in kilobases (kb).

Analysis of R_1 plants

As described previously [9], SC716 R_0 plants were backcrossed to nontransformed seed-derived B73 plants. Although pollen capable of germinating *in vitro* was recovered at a low frequency from SC716 R_0 plants [9], no R_1 plants were recovered using this pollen in backcrosses. Ears that developed on SC716 R_0 plants were pollinated with B73 pollen and viable R_1 plants were recovered from R_0 plants representing five independent, transgenic callus lines: T8, T18, T6/T11, T1 and T9 (Table 1). Fertility of R_0 plants was sporadic; progeny were recovered from only 35 of 219 R_0 plants (Table 1). Only a few kernels developed on each fertile R_0 plant; therefore, for analysis, R_1 plants were grouped into populations representing several R_0 plants and the transformed callus lines from which they were derived. R_1 plants were assayed for expression of *bar* by local foliar application of the herbicide Basta. We previously demonstrated that this is a reliable and sensitive method for detection of PAT activity in transgenic maize plants [9]. Segregation of PAT activity was observed in three R_1 populations: T6/T11, T8, and T18 (Table 1). No segregation was observed in the T9 R_1 population; all plants tested were tolerant to Basta application (Table 1). The single T1 R_1 plant was sensitive to Basta application (Table 1).

All of the SC716 callus lines that yielded PAT-

positive R_1 plants (T8, T18, T6/T11, and T9) were shown previously to have integrated *bar* and *uidA*. These four callus lines all expressed *bar* but lacked GUS activity [9]. Southern blot analyses were performed on R_1 plants derived from each callus line in order to confirm the presence of *bar* and *uidA* and to study segregation patterns and stability following meiosis. Southern blot analyses of *Eco* RI/*Hind* III-digested genomic DNA from eight T8 R_1 plants showed that the two *bar*-hybridizing fragments detected in T8 R_0 plants segregated together in the R_1 generation (Fig. 2A), indicating that all *bar*-hybridizing sequences were likely integrated on the same chromosome. As expected, PAT activity was detected only in R_1 plants containing *bar*-hybridizing sequence (Fig. 2A). To determine the segregation of *uidA* in T8 R_1 plants, *bar* probe was removed from the filter shown in Fig. 2A, and the filter was reprobed with *uidA*. The restriction enzymes used (*Eco* RI and *Hind* III) release a 2.1 kb fragment from pDPG208 containing *uidA* and the *nos* 3' end [9]. GUS-coding sequence cosegregated with *bar* in the T8 R_1 plants (Figs. 2A and B); all T8 R_1 plants that contained *bar*, also contained *uidA*-hybridizing sequence. However, not all *uidA*-hybridizing fragments detected in T8 R_0 plants were present in T8 R_1 plants (Fig. 2B). This may be due to segregation of multiple integrations of *uidA* on more than one chromosome or may be due to the loss of some *uidA* sequence. The lat-

Table 1. Segregation of PAT activity in R_1 populations recovered from backcrossed SC716 R_0 plants.

Callus line	R_0 plants	R_0 plants yielding viable progeny	Viable progeny recovered	PAT-positive progeny	PAT-negative progeny
T8	24	5	14	3	11
T18	17	6	34*	19	15
T5	3	0	0	—	—
T6/T11	94	4	7	2	5
T1	14	1	1	0	1
T4	2	0	0	—	—
T9	55	19	73	73	0
T17	10	0	0	—	—
T15	10	0	0	—	—

* Does not include three chlorophyll mutants.

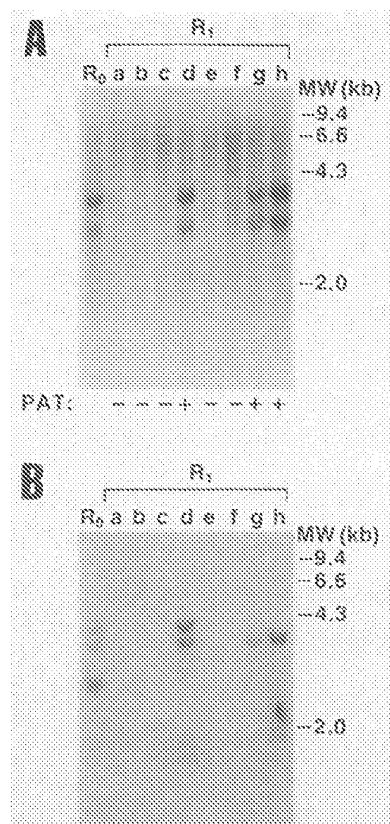


Fig. 2. Southern blot analyses of progeny (R_1) recovered from T8 R_0 plants. A. Genomic DNA from a T8 primary regenerative (R_0) and eight progeny (R_1) was digested with *Eco* RI and *Hind* III and probed with *bar*. R_1 plants a–d and e–h were recovered from separate R_0 plants. Presence (+) or absence (–) of PAT activity is indicated for each R_1 plant. B. The blot shown in A was stripped and reprobed with *uidA*.

ter seems more likely, given the fact that no hybridization to *uidA* was observed in plants that lacked *bar* (Figs. 2A and B).

Similar analyses of T18 R_1 plants showed that the two *bar*-hybridizing fragments present in *Eco* RI/*Hind* III-digested R_0 DNA segregated independently in the R_1 generation (Fig. 3A). T18 R_1 plants contained one, both or neither of the *bar*-hybridizing fragments present in T18 R_0 DNA. One of the fragments (ca. 6 kb) cosegregated with PAT activity (Fig. 3A), indicating that this fragment contained a functional copy of

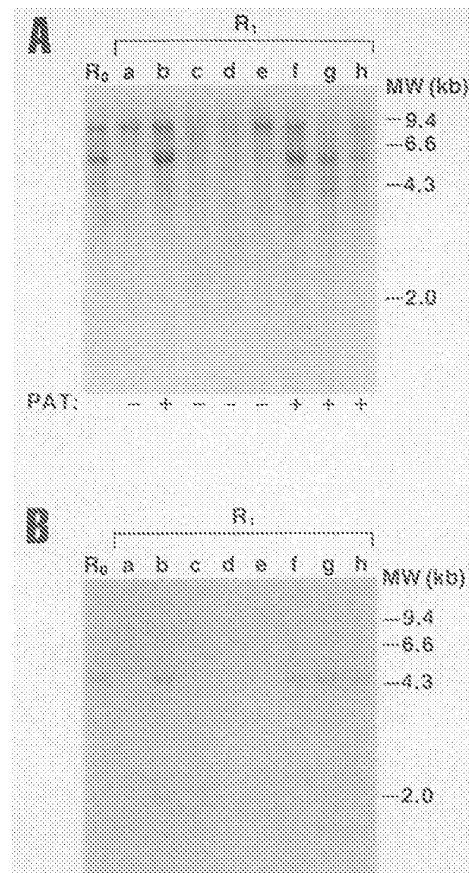


Fig. 3. Southern blot analyses of progeny (R_1) recovered from T18 R_0 plants. A. Genomic DNA from a T18 primary regenerative (R_0) and eight progeny (R_1) was digested with *Eco* RI and *Hind* III and probed with *bar*. R_1 plants a, b and c–h were recovered from separate R_0 plants. Presence (+) or absence (–) of PAT activity is indicated for each R_1 plant. B. The blot shown in A was stripped and reprobed with *uidA*.

bar while the larger-molecular-weight fragment (ca. 9 kb) was apparently nonfunctional. Reprobing the filter shown in Fig. 3A with *uidA* showed that the single *uidA*-hybridizing fragment present in T18 R_0 DNA cosegregated with the functional copy of *bar* (Fig. 3A and B).

Genomic DNA from the seven T6/T11 R_1 plants was digested with *Eco* RI and *Hind* III and analyzed by Southern blot hybridization using *bar* probe. Hybridization to high-molecular-weight (4–5 kb) fragments was observed for two T6/T11

R₁ plants, both of which had PAT activity (Fig. 4A). Segregation of the high-molecular-weight fragments was independent of the segregation of a smaller (ca. 1 kb), apparently non-functional *bar*-hybridizing fragment. Reprobing the filter shown in Fig. 4A with *uidA* probe revealed cosegregation of all *uidA* fragments present in T6/T11 R₀ DNA with the functional copy or copies of *bar* (Fig. 4A and B).

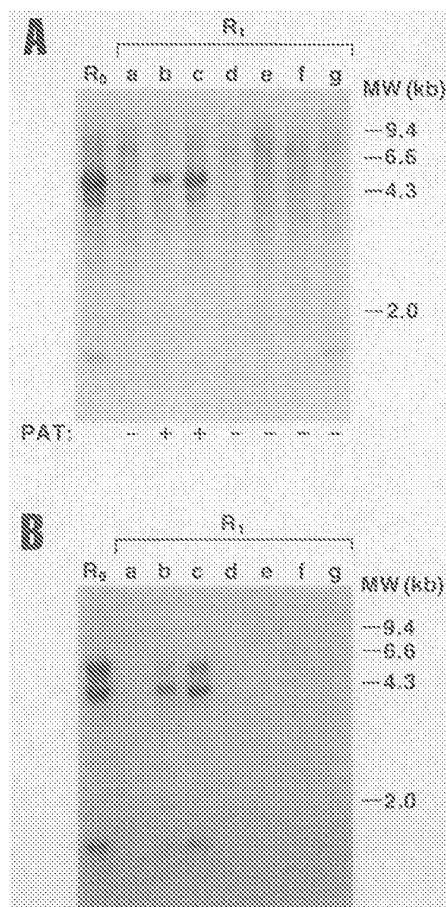


Fig. 4. Southern blot analyses of progeny (R₁) recovered from T6/T11 R₀ plants. A. Genomic DNA from a T6/T11 primary regenerant (R₀) and seven progeny (R₁) was digested with *Eco* RI and *Hind* III and probed with *bar*. R₁ plants a–c, d, e, f and g were recovered from four separate R₀ plants. Presence (+) or absence (–) of PAT activity is indicated for each R₁ plant. B. The blot shown in A was stripped and reprobed with *uidA*.

Southern blot analyses of *Eco* RI/*Hind* III-digested T9 R₁ genomic DNA demonstrated that the intact 1.9 kb *bar* expression unit fragment(s) present in T9 R₀ plants did not segregate and was inherited by all T9 R₁ plants tested (Fig. 5A). This is consistent with the lack of segregation for PAT activity observed in the T9 R₁ population (Table 1). To determine if the lack of *bar* segregation was a result of two or more integrations on homologous chromosomes, Southern blot analyses were performed on T9 R₀ and R₁ genomic DNA digested with either *Eco* RI or *Hind* III;

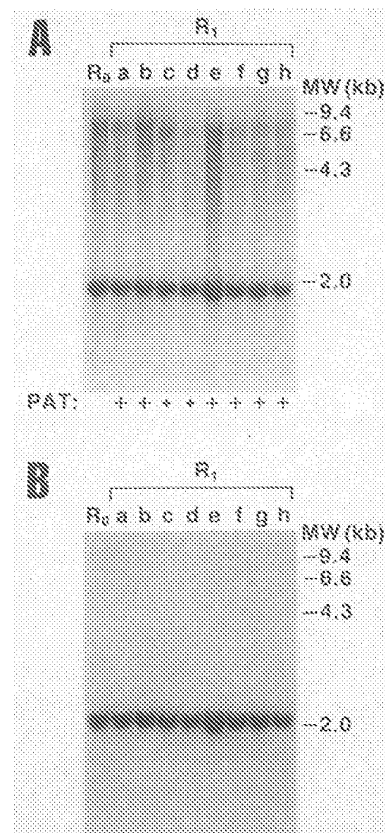


Fig. 5. Southern blot analyses of progeny (R₁) recovered from T9 R₀ plants. A. Genomic DNA from a T9 primary regenerant (R₀) and eight progeny (R₁) was digested with *Eco* RI and *Hind* III and probed with *bar*. R₁ plants a–d and e–h were recovered from separate R₀ plants. Presence (+) or absence (–) of PAT activity is indicated for each R₁ plant. B. The blot shown in A was stripped and reprobed with *uidA*.

both of these enzymes have unique sites on pDPG165 [9]. If multiple integrations had occurred, two or more fragments of varying size would be expected in T9 R₀ DNA, and these fragments would be expected to segregate in T9 R₁ plants. Only one fragment was observed for R₀ and R₁ plants using either *Eco* RI or *Hind* III (data not shown). Lack of segregation was also observed for *uidA* in T9 R₁ plants; reprobing the filter shown in Fig. 5A with *uidA* probe demonstrated the inheritance of a 2.1 kb *uidA*-hybridizing fragment by all T9 R₁ plants (Fig. 5B).

Although the incidence of fertility of backcrossed R₀ plants was low (Table 1), generalizations about the inheritance of the introduced genes can be made from the analysis of R₁ plants. Three fertile lines, T8, T18, and T6/T11, segregated for PAT activity and integrated plasmid DNA in the R₁ generation. However, the small number of T8, T18, and T6/T11 R₁ plants recovered precludes definitive conclusions about the modes of inheritance. Given the evidence that the functional copy or copies of *bar* segregate as a single unit in each of these lines, a 1:1 segregation for PAT activity would be expected in crosses with nontransformed plants, if sufficient numbers of progeny were recovered. In the T9 R₁ population, lack of segregation for PAT activity, *bar*, and *uidA*, as well as the evidence for a single *bar* integration event, suggest that either the transformation was nonnuclear (maternally inherited) or a single nuclear integration event was replicated in T9 callus yielding T9 R₀ plants that were homozygous for the introduced DNA.

Molecular analysis of the inheritance of foreign DNA in these four transgenic lines (T8, T18, T6/T11 and T9) was somewhat simplified by the fact that all four lines were the result of relatively low-copy-number transformations. Progeny expressing introduced plasmid DNA were not recovered from transgenic lines containing more than 5–6 copies of integrated plasmid DNA.

Analysis of R₂ plants

To further elucidate the modes of inheritance of introduced DNA in the transgenic lines, PAT-

positive T8, T9, T6/T11, and T18 R₁ plants were crossed to nontransformed inbred plants. The fertility of R₁ plants was substantially improved over that of R₀ plants; viable R₂ plants were recovered from all four lines using PAT-positive R₁ plants as either the female or male parent. Seed-set as the result of R₁ crosses, regardless of the direction of the cross, was usually normal. In a previous report [10], we described dramatic improvements in the fertility of transgenic maize lines after one or two backcrosses. We do not, as yet, have any evidence to suggest that integration or expression of foreign genes affects fertility or plant vigor.

Although callus lines T8, T9, T6/T11, and T18 all lacked GUS activity [9], molecular analyses of R₁ plants demonstrated that *uidA* was present and apparently linked to functional *bar* in all four lines (Figs. 2, 3, 4, 5). R₂ plants were assayed fluorometrically [12] for GUS activity to determine if expression was differentially regulated in callus and plants. Several R₂ plants from each line were tested; all were negative (data not shown).

PAT activity in R₂ plants was determined by spraying seedlings at the 2–3 leaf stage with a 1% (v/v) Basta solution. For each line, numerous R₂ progeny of single PAT-positive R₁ plants were



Fig. 6. Herbicide application to T18 R₂ plants segregating for *bar*. The plant on the left was sprayed with water and four plants on the right were sprayed with a 1% (v/v) Basta solution. The photograph was taken seven days after herbicide application.

Table 2. Analysis of PAT activity segregation in R₂ plants derived from crosses of transgenic R₁ plants with nontransformed inbred plants.

R ₁ parent	Cross	R ₂ plants sprayed with Basta		Expected ratio	χ^2 ^a	<i>P</i> ^b
		Resistant	Sensitive			
T8	male	0	36	1:1	34.02	<0.001
T8	female	2	34	1:1	26.69	<0.001
T9	male	15	15	1:1	0.03	0.70
T9	female	13	17	1:1	0.30	0.50
T6/T11	male	24	30	1:1	0.46	0.50
T6/T11	female	31	23	1:1	0.91	0.30
T18	male	31	29	1:1	0.02	0.90
T18	female	33	27	1:1	0.42	0.50

^a χ^2 values calculated using the Yates correction term as described by Strickberger [20].

^b *P* = χ^2 probability with 1 d.f.

analyzed, half derived from a backcross using the R₁ as the female, and half using the R₁ as the male. PAT activity was easily assessed; sensitive plants began to appear necrotic two to three days after herbicide application and were completely necrotic after one week, while most resistant plants were indistinguishable from water-sprayed controls (Fig. 6). Occasionally, small lesions appeared on leaf tips or at the base of the whorl of resistant plants, however plant vigor was unaffected. Applications of up to 21% (v/v) Basta were tested on T18 R₂ plants. Some small lesions and leaf cupping were occasionally observed 1–2 weeks after application on resistant plants, but subsequent growth and fertility were unaffected (data not shown).

As assayed by Basta application, PAT activity segregated 1:1 in the R₂ generations derived from individual transgenic T9, T6/T11, and T18 R₁ plants (Table 2). Recovery of Basta-resistant T8 R₂ plants was sporadic (Table 2). Results from additional Basta applications to R₂ plants recovered from several transgenic R₁ plants from each line were consistent with data presented in Table 2 (data not shown). One-to-one segregation of functional *bar* in T18 and T6/T11 R₂ plants is consistent with molecular and enzyme activity analyses of R₁ populations (Figs. 3 and 4). The reason for the low frequency of PAT-positive T8 R₂ plants was investigated by performing South-

ern analyses on twelve T8 R₂ plants. The twelve R₂ plants, including one PAT-positive plant, were all derived from a single PAT-positive T8 R₁ plant. If gene inactivation were responsible for the low frequency of PAT-positive T8 R₂ plants, some of the PAT-negative T8 R₂ plants would be expected to contain *bar*. Southern analyses revealed the presence of *bar*-hybridizing fragments in only the single PAT-positive R₂ plant (data not shown). These data indicated that loss of the gene, or poor transmission of the gene was responsible for the aberrant segregation ratio of T8 R₂ plants (Table 2). Given the molecular evidence for the loss of some *uidA* sequence in T8 R₁ plants (Fig. 2B), instability of the T8 integration event seems likely. It is also possible that the T8 integration event was linked to a deleterious chromosome abnormality such as a small duplication or deletion and therefore transmitted at a low frequency. Segregation of PAT activity in T9 R₂ plants (Table 2), coupled with the molecular evidence for a single integration event, supports the hypothesis that a single integration event was replicated in T9 callus, yielding T9 R₀ plants that were homozygous for *bar* and *uidA*. This finding also rules out the possibility of a nonnuclear transformation event. Possible mechanisms responsible for this homozygosity include mitotic recombination [19] and gene conversion [14]. Gene conversion is a particularly attractive ex-

planation, given the possibility that integration of plasmid DNA and the replication event may have been concomitant, both the result of the same nick in chromosomal DNA.

Conclusions

Data in this report demonstrate Mendelian inheritance of a functional gene introduced into maize by microprojectile bombardment. Functional *bar* sequence(s) segregated as single units in all four transgenic lines analyzed; however, integrated copies of *bar* appeared to be unstable or poorly transmitted in one line. Independent segregation of apparently nonfunctional copies of *bar* was also observed. GUS-coding sequence, introduced simultaneously on a separate plasmid, was inherited by all four lines and appeared to be linked to functional *bar* in all cases. However, expression of *uidA* was not detected in any of the lines described here. These lines were recovered from experiments using separate plasmids encoding the selectable and nonselectable markers. Coexpression frequencies in these experiments, analyzed at the callus level, were approximately 20% [9]. Currently, we are improving coexpression frequency by bombarding with single plasmids encoding both a selectable and a nonselectable marker.

Using microprojectile bombardment, we can now study the processes involved in heterologous gene function in maize. An increased understanding of the mechanisms of gene integration, as well as the effects of gene position and copy number on expression, should facilitate significant genetic manipulation of maize in the future.

Acknowledgements

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11/587,893

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L1	5 S ARYLOXYALKANOATE DIOXYGENASE
L2	57 S TAURINE DIOXYGENASE
L3	1 S L2 AND PLANT
L4	48 S AAD (A) 1
L5	41 DUP REM L4 (7 DUPLICATES REMOVED)
L6	3 S L5 AND PLANT
L7	3 S TDFA AND PLANT
L8	2 DUP REM L7 (1 DUPLICATE REMOVED)



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CONFIRMATION NO. 7868

SERIAL NUMBER 11/587,893	FILING or 371(c) DATE 05/22/2008 RULE	CLASS 800	GROUP ART UNIT 1638	ATTORNEY DOCKET NO. DAS-P0115-US-02		
APPLICANTS Terry R. Wright, Westfield, IN; Justin M. Lira, Fishers, IN; Donald J. Merlo, Carmel, IN; Nicole L. Arnold, Carmel, IN;						
** CONTINUING DATA ***** This application is a 371 of PCT/US2005/014737 05/02/2005 which claims benefit of 60/567,052 04/30/2004 /DK/						
** FOREIGN APPLICATIONS ***** NONE /DK/						
** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** 12/19/2008						
Foreign Priority claimed ☐ Yes ☒ No 35 USC 119(a-d) conditions met ☐ Yes ☒ No Verified and /DAVID H KRUSE/ Acknowledged Examiner's Signature		☐ Met after Allowance /DK/ Initials	STATE OR COUNTRY IN	SHEETS DRAWINGS 24	TOTAL CLAIMS 67	INDEPENDENT CLAIMS 4
ADDRESS Baker & Daniels LLP- Dow AgroSciences 300 North Meridian Street, Suite 2700 Indianapolis, IN 46204 UNITED STATES						
TITLE Novel Herbicide Resistance Genes						
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Application Number 11/587,893		Filed May 22, 2008	
For Novel Herbicide Resistance Genes			
Art Unit 1638		Examiner David H. Kruse	
This is a request under the provisions of 37 CFR 1.136(a) to extend the period for filing a reply in the above identified application.			
The requested extension and fee are as follows (check time period desired and enter the appropriate fee below):			
	<u>Fee</u>	<u>Small Entity Fee</u>	
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☐ Applicant claims small entity status. See 37 CFR 1.27.			
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July 8, 2010

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants : Terry Wright, Justin Lira, Donald Merlo, Nicole Hopkins
Docket No. : DAS-P0115-US-02
Title : Novel Herbicide Resistance Genes
Serial No. : 11/587,893

RESPONSE

In response to the office action dated March 9, 2010, reconsideration is respectfully requested.

Authorization for a one-month extension of time accompanies this response.

Please amend the above-identified patent application as follows:

In the Claims

1 (currently amended). A transgenic plant cell comprising a recombinant polynucleotide that encodes an AAD-1 protein comprising the amino acid sequence of SEQ ID NO:9 or a variant of SEQ ID NO:9 wherein said variant has aryloxyalkanoate dioxygenase activity, at least one amino acid deletion or conserved substitution, and at least 95% sequence identity with SEQ ID NO:9.

2 (original). The cell of claim 1 wherein said plant cell is selected from the group consisting of dicotyledonous cells and monocotyledonous cells.

3 (original). The cell of claim 2 wherein said plant cell is dicotyledonous and selected from the group consisting of a cotton cell, a tobacco cell, a canola cell, a soybean cell, and an *Arabidopsis* cell.

4 (original). The cell of claim 2 wherein said plant cell is a monocotyledonous cell selected from the group consisting of a rice cell and a maize cell.

5 (original). A transgenic plant comprising a plurality of cells according to claim 1, wherein expression of said polynucleotide renders said cells tolerant to an aryloxyalkanoate herbicide.

6 (original). The plant of claim 5 wherein said herbicide is a phenoxy auxinic herbicide.

7 (original). The plant of claim 5 wherein said herbicide is selected from the group consisting of 2,4-dichlorophenoxyacetic acid, MCPA, dichlorprop, and mecoprop.

8 (original). The plant of claim 5 wherein said herbicide is an aryloxyphenoxypropionate.

9 (original). The plant of claim 5 wherein said herbicide is selected from the group consisting of fluazifop, haloxyfop, diclofop, quizalofop, fenoxaprop, metamifop, cyhalofop, and clodinofop.

10 (original). The plant of claim 5 wherein expression of said polynucleotide renders said plant resistant to both a phenoxy auxin herbicide and an aryloxyphenoxypropionate herbicide.

11 (original). The plant of claim 5 wherein said plant further comprises a second herbicide resistance gene.

12 (original). The plant of claim 11 wherein said second herbicide resistance gene renders said plant resistant to a herbicide selected from the group consisting of glyphosate, glufosinate, ALS inhibitors, inhibitors of 4-hydroxyphenyl-pyruvate-dioxygenase (HPPD), and inhibitors of protoporphyrinogen oxidase (PPO).

13 (original). A method of controlling at least one weed in a field, wherein said field contains at least one plant of claim 5, wherein said method comprises applying to at least a portion of said field a first herbicide selected from the group consisting of a phenoxy auxin herbicide and an aryloxyphenoxypropionate herbicide.

14 (original). The method of claim 13 wherein said herbicide is an R-enantiomer of a chiral phenoxy auxin.

15 (original). The method of claim 14 wherein said herbicide is selected from the group consisting of R-dichlorprop and R-mecoprop.

16 (original). The method of claim 13 wherein said herbicide is an achiral phenoxy auxin.

17 (original). The method of claim 16 wherein said herbicide is selected from the group consisting of 2,4-D and MCPA.

18 (original). The method of claim 13 wherein said first herbicide is an aryloxyphenoxypropionate and said plant is a monocot.

19 (original). The method of claim 18 wherein said monocot is selected from the group consisting of corn, rice, wheat, barley, rye, turf grass, oats, sorghum, and pasture grasses.

20 (original). The method of claim 13 wherein said first herbicide is a phenoxy auxin and said plant is a dicot.

21 (original). The method of claim 20 wherein said dicot is selected from the group consisting of cotton, tobacco, canola, and soybean.

22 (original). The method of claim 13 wherein said method comprises applying a second herbicide.

23 (original). The method of claim 22 wherein said first herbicide and said second herbicide are applied sequentially.

24 (original). The method of claim 22 wherein said first herbicide and said second herbicide are applied concurrently.

25 (original). The method of claim 22 wherein said first herbicide is a phenoxy auxin and second herbicide is an aryloxyphenoxypropionate.

26 (original). The method of claim 13 wherein said plant is resistant to glyphosate.

27 (original). The method of claim 14 wherein said R-enantiomer is a component of a racemic mixture.

28 (original). The method of claim 22 wherein said plant further comprises a second herbicide resistance gene that renders said plant resistant to said second herbicide.

29 (original). The method of claim 28 wherein said second gene is selected from the group consisting of a modified AHAS (acetohydroxyacid synthase), SurA, SurB, Csr1, Csr1-1, Csr1-2, a modified EPSPS (5-enolpyruvylshikimate-3-phosphate synthase), GOX, GAT, PAT (phosphinothricin-N-acetyltransferase), bar, and a dicamba-degrading enzyme.

30 (original). The method of claim 28 wherein said second herbicide is selected from the group consisting of glyphosate, glufosinate, dicamba, acetolactate synthase inhibitors, protoporphyrinogen oxidase inhibitors, and hydroxyphenyl-pyruvate-dioxygenase inhibitors.

31 (original). The method of claim 30 wherein said second herbicide is an acetolactate synthase inhibitor selected from the group consisting of imidazolinones, sulfonyl ureas, and triazolopyrimidine herbicides.

32 (original). The method of claim 31 wherein said second herbicide is an imidazolinone selected from the group consisting of imazamox, imazethapyr, imazaquin, and imazapic.

33 (original). The method of claim 28 wherein said first herbicide is a phenoxy auxin and said second herbicide is selected from the group consisting of glyphosate and glufosinate.

34 (original). The method of claim 33 wherein said phenoxy auxin is 2,4-D and said second herbicide is glyphosate.

35 (original). The method of claim 28 wherein said first herbicide is an aryloxyphenoxypropionate and said second herbicide is glyphosate.

36 (original). The method of claim 35 wherein said first herbicide is selected from the group consisting of quizalofop, haloxyfop, and cyhalofop.

37 (original). The method of claim 31 wherein said second herbicide is a sulfonyl urea selected from the group consisting of amidosulfuron, bensulfuron, chlorimuron, chlorsulfuron, cinosulfuron, flupyrsulfuron, foramsulfuron, halosulfuron, nicosulfuron, primisulfuron, prosulfuron, rimsulfuron, sulfometuron, sulfosulfuron, thifensulfuron, triasulfuron, trifloxysulfuron, and triflusulfuron.

38 (original). The method of claim 28 wherein said method further comprises applying a third herbicide.

39 (original). The method of claim 38 wherein said herbicides are 2,4-D; quizalofop; and glufosinate.

40 (currently amended). A method of controlling weeds in a field, wherein said method comprises applying a first herbicide to said field and planting a seed in said field within 14 days of applying said first herbicide, wherein said seed comprises ~~a~~ the cell of claim 1, and wherein said first herbicide is selected from the group consisting of a phenoxy auxin and an aryloxyphenoxypropionate.

41 (original). The method of claim 40 wherein said first herbicide is an acid, an inorganic salt, an organic salt, an ester, an R-enantiospecific isomer, or a component of a racemic mixture.

42 (original). The method of claim 40 wherein said seed comprises a second gene that renders said plant resistant to a second herbicide, and said method further comprises applying said second herbicide to said field prior to said planting.

43 (original). The method of claim 42 wherein said second herbicide is selected from the group consisting of glyphosate, gramoxone, and glufosinate

44 (currently amended). A polynucleotide optimized for expression in a plant wherein said polynucleotide encodes a protein having aryloxyalkanoate dioxygenase activity, wherein ~~a nucleic acid molecule that encodes said protein~~ is at least 95% identical with a protein encoded by ~~hybridizes under stringent conditions with the full complement of a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5.~~

45 (original). The polynucleotide of claim 44 wherein said polynucleotide is optimized for expression in a dicotyledonous plant or a monocotyledonous plant.

46 (original). The polynucleotide of claim 44 wherein said polynucleotide comprises SEQ ID NO:5.

47 (currently amended). An isolated polynucleotide that encodes a protein that enzymatically degrades a herbicide selected from the group consisting of a phenoxy auxin and an aryloxyphenoxypionate, wherein ~~a nucleic acid molecule that encodes said protein~~ is at least 95% identical with ~~hybridizes under stringent conditions with the full complement of a sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:4, and SEQ ID NO:5, SEQ ID NO:9, SEQ ID NO:10, and SEQ ID NO:11,~~ wherein said polynucleotide is operably linked to a promoter that is functional in a plant cell.

48 (original). The polynucleotide of claim 47 wherein said promoter is a plant promoter.

49 (original). The polynucleotide of claim 47 wherein said promoter is a cassava vein mosaic virus promoter.

50 (currently amended). A method of selecting for a transformed plant cell wherein said method comprises subject a plurality of plant cells to transformation with ~~a~~the polynucleotide of claim 47 and growing said cells in a concentration of a herbicide that permits transformed cells that express said polynucleotide to grow while killing or inhibiting the growth of nontransformed cells, wherein said herbicide is selected from the group consisting of a phenoxy auxin and an aryloxyphenoxyalkanoate.

51 (original). The method of claim 50 wherein said cells are cells of a plant and said method is used for selecting a transformed plant.

52-56 (canceled).

57 (currently amended). A seed comprising ~~a~~the plant cell of claim 1.

58 (original). A plant grown from the seed of claim 57.

59 (currently amended). A part, progeny, or asexual propagate of the plant of claim 5, wherein said part, progeny, or sexual propagate comprises said polynucleotide.

60 (currently amended). The method of claim 13 wherein said method is used to treat or prevent herbicide-resistant weeds, wherein said herbicide-resistant weeds are sensitive to a herbicide selected from the group consisting of a phenoxy auxin herbicide and an aryloxyphenoxypropionate herbicide.

61 (original). The plant of claim 5 wherein said plant further comprises an insect-resistance gene derived from an organism selected from the group consisting of *Bacillus thuringiensis*, *Photorhabdus*, and *Xenorhabdus*.

62 (original). The plant of claim 5 wherein said plant further comprises a gene for an agronomic trait selected from the group consisting of fungal resistance, stress tolerance, increased yield, improved oil profile, improved fiber quality, viral resistance, delayed ripening, cold tolerance, and salt tolerance.

63 (canceled).

64 (original). A method of controlling glyphosate-resistant weeds in a field of glyphosate tolerant crop plants, wherein said plants comprise a polynucleotide of claim 47, and said method comprises applying an aryloxyalkanoate herbicide to at least a portion of said field.

65 (original). The method of claim 64 wherein said herbicide is a phenoxy auxin.

66 (original). The method of claim 64 wherein said herbicide is an aryloxy phenoxypropionate.

67 (original). The method of claim 64 wherein said plants are dicots.

68 (original). The method of claim 64 wherein said plants are monocots.

Remarks

Claims 1-51 and 53-68 were the subject of the office action dated March 9, 2010.

Claims 53-56 were withdrawn from consideration as being non-elected. These claims are canceled accordingly without prejudice. Claim 63 is also canceled for consistency in favor of the other claims.

Thus, claims 1-51 and 57-68 are now presented for further consideration.

Claims 44 and 47 are amended to be consistent with claim 1.

Claims 1, 40, 50, and 57 are amended as suggested on pages 2-3 of the office action.

Claim 60 is amended to clarify that herbicide-resistant weeds would be sensitive to the herbicides specified in claim 60. Sensitive plants and herbicide-resistant weeds are discussed in paragraphs 7, 9, 11, 17, and throughout the Examples. Paragraphs 6-7 and 79-80 and Examples 19-21 discuss concern about glyphosate-resistant weeds in the context of glyphosate-only weed control programs. Example 22 discusses preventing glufosinate-resistant weeds. Example 23 discusses imidazolinone resistance prevention.

As discussed therein, there is concern that relying on only a single herbicide (such as glyphosate) for weed control could select for weeds that are resistant to that herbicide. Thus, the subject herbicides (phenoxy auxins (such as 2,4-D; 2,4-DB; 2,4-dichlorophenoxypropionates; MCPA; and MCPB) and aryloxyphenoxypropionates) can be used to kill weeds that would otherwise grow to develop resistance to glyphosate, for example. The subject herbicides can be used in fields of crops that comprise the AAD-1 genes without significant injury to the crop plants – while controlling sensitive weeds and any glyphosate-resistant weeds, for example. Crops can be "stacked" with a subject AAD-1 gene and a glyphosate-resistance gene, so that both types of herbicides could be used without significant crop injury.

The written description rejection of claims 44-51 and 63-68 should be rendered moot. Original language from claim 1 is now also used in claims 44 and 47.

Claim 59 is amended to clarify that the progeny, parts, or propagates comprise the polynucleotide of claim 1. Thus, the obviousness rejection of this claim should be rendered moot.

Claims 1-51 and 57-68 stand rejected as being obvious in view of Kaphammer (U.S. Patent No. 5,608,147), Kohler (1999), van der Meer (2004), and Jepson (US 2003/0041357). The applicants respectfully traverse this rejection.

Kaphammer is cited as teaching plants and plant cells transformed with a *tfdA* gene. The examiner admits that Kaphammer does not teach a polynucleotide that is at least 95% identical to SEQ ID NO:9.

van der Meer is cited as teaching SEQ ID NO:9 from *Sphingobium herbicidovorans*.

Jepson is cited as teaching the introduction of transgenes into plants.

There are no specific comments regarding Kohler in the office action. Kohler is entitled "*Sphingomonas herbicidovorans* MH: a versatile phenoxyalkanoic acid herbicide degrader." The office action simply states that given the teachings of Kohler, one would have had a reasonable expectation of success to produce a plant that is resistant to aryloxyphenoxypropionates. However, the abstract of Kohler states:

Sphingomonas herbicidovorans MH was isolated from a dichlorprop-degrading soil column. It is able to grow on phenoxyalkanoic acid herbicides, such as mecoprop, dichlorprop, 2,4-D, MCPA, and 2,4-DB. Strain MH utilizes both enantiomers of the chiral herbicides mecoprop and dichlorprop as sole carbon and energy sources. Enantiomer-specific uptake systems are responsible for transporting the acidic substrates across the cell membrane. Catabolism is initiated by two enantiomer-specific α -ketoglutarate-dependent dioxygenases that catalyze the cleavage of the ether bond of the respective enantiomer to yield the corresponding phenol and pyruvate. Therefore selective degradation of the enantiomers of mecoprop and dichlorprop by strain MH is not only due to enantioselective catabolism but also to enantioselective transport.

As illustrated by Tables 1 and 2 in the subject specification, mecoprop and dichlorprop have phenoxy auxin chemistries, while the aryloxyphenoxypropionates or "fops" are in a different class of chemistries. Thus, it is not seen how Kohler would support the reasonable expectation of success to produce a plant that is resistant to aryloxyphenoxypropionates.

Moreover, the subject AAD-1 gene was a choice of one of over 100 other possible genes.

Example 1 (paragraph 179) of the subject application (as published) states [emphasis added]:

As a way to identify genes which possess herbicide degrading activities in planta, it is possible to mine current public databases such as NCBI (National Center for Biotechnology Information). To begin the process, it is necessary to have a functional gene sequence already identified that encodes a protein with the desired characteristics (i.e., .alpha.-ketoglutarate dioxygenase activity). This protein sequence is then used as the input for the BLAST (Basic Local Alignment Search Tool) (Altschul et al, 1997) algorithm to compare against available NCBI protein sequences deposited. Using default settings, this search returns upwards of 100 homologous protein sequences at varying levels. These range from highly identical (85-98%) to very low identity (23-32%) at the amino acid level. Traditionally only sequences with high homology would be expected to retain similar properties to the input sequence. In this case we chose only those sequences with <50% homology. We go on to exemplify that cloning and recombinantly expressing homologues with as little as 27% amino acid conservation can be used to impart commercial levels of resistance not only to the intended herbicide, but also to substrates never previously tested with these enzymes.

Thus, the subject AAD-1 gene was only 1 of over 100 BLAST search results having some degree of identity with tfdA. As discussed above, there were a great number of other possible choices to begin with.

In addition, the behavior and activity of a herbicide-tolerance gene in bacteria cannot be predicted in plants. This is illustrated by, for example, Example 17 of the subject specification, which compares the subject AAD-1 genes to another gene referred to as AAD-2. AAD-2 was another gene identified from BLAST search results. Paragraph 444 of the published application (paragraph 434 of the application as filed) states:

Another gene was identified from the NCBI database (see the ncbi.nlm.nih.gov website; accession #AP005940) as a homologue with only 44% amino acid identity to tfdA. This gene is referred to herein as AAD-2 (v1) for consistency. Percent identity was determined by first translating both the AAD-2 and tfdA DNA sequences (SEQ ID NO:12 and GENBANK Accession No. M16730, respectively) to proteins (SEQ ID NO:13 and GENBANK Accession No. M16730, respectively), then using ClustalW in the Vector NTI software package to perform the multiple sequence alignment.

AAD-2 was obtained from *Bradyrhizobium japonicum*. Again, AAD-2 is more related (about 44%) at the sequence level to tfdA than is AAD-1 (about 27%).

AAD-2 was more active than AAD-1 *in vitro* using 2,4-D as a substrate. However, AAD-2 was surprisingly inactive in plants, while AAD-1 was very active. For example, paragraph 452 states:

The enzyme kinetics of partially purified AAD-1 (v1) and AAD-2 (v1) were compared using 2,4-D as substrate. See FIG. 19. The K_m values for 2,4-D were 97 and 423 μM for AAD-1 (v1) and AAD-2 (v1) respectively and the apparent V_{max} values were 0.11 and 0.86 A510 units, respectively (Table 34). Because equivalent amounts of enzyme were used in the assays (as determined by SDS-PAGE analysis), it can be concluded that the k_{cat} of AAD-2 (v1) for 2,4-D is almost 8-fold higher than AAD-1 (v1) and the k_{cat}/K_m is 2-fold higher. Thus AAD-2 (v1) is significantly more efficient at cleaving 2,4-D *in vitro* than AAD-1 (v1). This is in surprising contrast to the *in planta* findings reported below, where plants expressing AAD-1 (v1) are significantly better in conferring 2,4-D resistance relative to AAD-2 (v1).

This shows that herbicidal activity in plants is unpredictable and that the results of van der Meer could not readily be applied to *in planta* behavior. Furthermore, there were over a hundred other choices to begin with; AAD-1 and AAD-2 were choices among more than a hundred. If AAD-2 were used as the point of reference (rather than the gene of van der Meer), one would predict no activity in plants for bacterial genes.

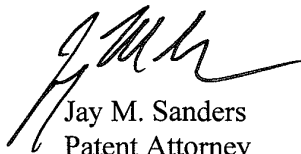
The foregoing should show that there was no reasonable expectation of successfully producing a herbicide-tolerant plant using AAD-1 (or AAD-2). In light of this, the withdrawal of the subject rejection for obviousness is respectfully requested.

Regarding the provisional obviousness-type double-patenting rejection, the exemplified AAD-13 protein of USSN 12/599,385 shares only about 27% identity with the exemplified AAD-1 protein of the subject application. Thus, it is unclear how claims 99-126 of that application would render claims 1-51 and 57-68 of the subject application obvious, and vice versa. At only 27% identity, it is not seen how hybridization could be maintained between genes encoding AAD-1 proteins and AAD-13 proteins, as stringent hybridization conditions are defined in the specification of USSN 12/599,385 as being, for example, 1X SSPE at room temperature. Thus, no overlap in scope is apparent to the applicants, and the withdrawal of this provisional rejection is respectfully requested.

The subject application is believed to be in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees as required by this paper to Deposit Account No. 02-0390.

Respectfully submitted,



Jay M. Sanders

Patent Attorney

Registration No. 39,355

Phone No.: 317-237-1245

Fax No.: 317-237-1000

Address: BAKER & DANIELS LLP
300 N. Meridian Street
Suite 2700
Indianapolis, IN 46204

Electronic Patent Application Fee Transmittal				
Application Number:		11587893		
Filing Date:		22-May-2008		
Title of Invention:		Novel Herbicide Resistance Genes		
First Named Inventor/Applicant Name:		Terry R. Wright		
Filer:		Jay M. Sanders/Lisa Schodrowski		
Attorney Docket Number:		DAS-P0115-US-02		
Filed as Large Entity				
U.S. National Stage under 35 USC 371 Filing Fees				
Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Basic Filing:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Extension - 1 month with \$0 paid	1251	1	130	130

Description	Fee Code	Quantity	Amount	Sub-Total in USD(\$)
Miscellaneous:				
Total in USD (\$)				130

Electronic Acknowledgement Receipt	
EFS ID:	7976971
Application Number:	11587893
International Application Number:	
Confirmation Number:	7868
Title of Invention:	Novel Herbicide Resistance Genes
First Named Inventor/Applicant Name:	Terry R. Wright
Customer Number:	83067
Filer:	Jay M. Sanders/Lisa Schodrowski
Filer Authorized By:	Jay M. Sanders
Attorney Docket Number:	DAS-P0115-US-02
Receipt Date:	08-JUL-2010
Filing Date:	22-MAY-2008
Time Stamp:	16:14:55
Application Type:	U.S. National Stage under 35 USC 371

Payment information:

Submitted with Payment	yes
Payment Type	Deposit Account
Payment was successfully received in RAM	\$ 130
RAM confirmation Number	2655
Deposit Account	020390
Authorized User	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
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1	Extension of Time	DAS-P0115-Extension-Time. PDF	73745 bfb825eb44228860988846cea11359fcd 4d053	no	1
Warnings:					
Information:					
2		DAS-P0115-Response.PDF	506718 ee1e0b8fcdabd0e06b29f005b038b7e74d 9de40	yes	14
	Multipart Description/PDF files in .zip description				
	Document Description		Start	End	
	Applicant Arguments/Remarks Made in an Amendment		1	1	
	Claims		2	9	
	Applicant Arguments/Remarks Made in an Amendment		10	14	
Warnings:					
Information:					
3	Fee Worksheet (PTO-875)	fee-info.pdf	30386 a97c655e72988f8405d30bf0c3acc8ff8f78 33b	no	2
Warnings:					
Information:					
Total Files Size (in bytes):			610849		
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875					Application or Docket Number 11/587,893		Filing Date 05/22/2008		☐ To be Mailed	
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APPLICATION AS FILED – PART I						OTHER THAN SMALL ENTITY				
(Column 1)		(Column 2)		SMALL ENTITY ☐		OR		SMALL ENTITY		
FOR	NUMBER FILED	NUMBER EXTRA	RATE (\$)	FEE (\$)	OR	RATE (\$)	FEE (\$)	OR	RATE (\$)	FEE (\$)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A	N/A			N/A			N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (l), or (m))	N/A	N/A	N/A			N/A			N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A	N/A			N/A			N/A	
TOTAL CLAIMS (37 CFR 1.16(j))	minus 20 =	*	X \$	=		X \$	=		X \$	=
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*	X \$	=		X \$	=		X \$	=
☐ APPLICATION SIZE FEE (37 CFR 1.16(s))	If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$250 (\$125 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s).									
☐ MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))										
* If the difference in column 1 is less than zero, enter "0" in column 2.						TOTAL			TOTAL	

APPLICATION AS AMENDED – PART II						OTHER THAN SMALL ENTITY					
(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR		SMALL ENTITY	
AMENDMENT	07/08/2010	CLAIMS REMAINING AFTER AMENDMENT	Minus	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	OR	RATE (\$)	ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))	* 62	Minus	** 67	= 0	X \$	=		X \$52=	0	
	Independent (37 CFR 1.16(h))	* 4	Minus	***4	= 0	X \$	=		X \$220=	0	
☐ Application Size Fee (37 CFR 1.16(s))											
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))											
						TOTAL ADD'L FEE			TOTAL ADD'L FEE	0	

(Column 1)		(Column 2)		(Column 3)		SMALL ENTITY		OR		SMALL ENTITY	
AMENDMENT	Total (37 CFR 1.16(i))	CLAIMS REMAINING AFTER AMENDMENT	Minus	HIGHEST NUMBER PREVIOUSLY PAID FOR	PRESENT EXTRA	RATE (\$)	ADDITIONAL FEE (\$)	OR	RATE (\$)	ADDITIONAL FEE (\$)	
	Total (37 CFR 1.16(i))	*	Minus	**	=	X \$	=		X \$	=	
	Independent (37 CFR 1.16(h))	*	Minus	***	=	X \$	=		X \$	=	
☐ Application Size Fee (37 CFR 1.16(s))											
☐ FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))											
						TOTAL ADD'L FEE			TOTAL ADD'L FEE		

* If the entry in column 1 is less than the entry in column 2, write "0" in column 3.
 ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".
 *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".
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Legal Instrument Examiner:
/DOROTHY BELL/

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

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NOTICE OF ALLOWANCE AND FEE(S) DUE

83067 7590 09/24/2010
Baker & Daniels LLP- Dow AgroSciences
300 North Meridian Street, Suite 2700
Indianapolis, IN 46204

EXAMINER

KRUSE, DAVID H

ART UNIT

PAPER NUMBER

1638

DATE MAILED: 09/24/2010

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/587,893	05/22/2008	Terry R. Wright	DAS-P0115-US-02	7868

TITLE OF INVENTION: NOVEL HERBICIDE RESISTANCE GENES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1510	\$300	\$0	\$1810	12/27/2010

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

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If the SMALL ENTITY is shown as YES, verify your current SMALL ENTITY status:

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B. If applicant claimed SMALL ENTITY status before, or is now claiming SMALL ENTITY status, check box 5a on Part B - Fee(s) Transmittal and pay the PUBLICATION FEE (if required) and 1/2 the ISSUE FEE shown above.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed and an extra copy of the form should be submitted. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Utility patents issuing on applications filed on or after Dec. 12, 1980 may require payment of maintenance fees. It is patentee's responsibility to ensure timely payment of maintenance fees when due.

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** **Mail Stop ISSUE FEE**
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CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

83067 7590 09/24/2010
Baker & Daniels LLP- Dow AgroSciences
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Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

(Depositor's name)
(Signature)
(Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/587,893	05/22/2008	Terry R. Wright	DAS-P0115-US-02	7868

TITLE OF INVENTION: NOVEL HERBICIDE RESISTANCE GENES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1510	\$300	\$0	\$1810	12/27/2010

EXAMINER	ART UNIT	CLASS-SUBCLASS
KRUSE, DAVID H	1638	800-300000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).
☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list
 (1) the names of up to 3 registered patent attorneys or agents OR, alternatively, _____ 1
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3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE _____ (B) RESIDENCE: (CITY and STATE OR COUNTRY) _____

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

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5. Change in Entity Status (from status indicated above)

- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

NOTE: The Issue Fee and Publication Fee (if required) will not be accepted from anyone other than the applicant; a registered attorney or agent; or the assignee or other party in interest as shown by the records of the United States Patent and Trademark Office.

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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/587,893	05/22/2008	Terry R. Wright	DAS-P0115-US-02	7868
83067	7590	09/24/2010	EXAMINER	
Baker & Daniels LLP- Dow AgroSciences 300 North Meridian Street, Suite 2700 Indianapolis, IN 46204			KRUSE, DAVID H	
			ART UNIT	PAPER NUMBER
			1638	
DATE MAILED: 09/24/2010				

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment to date is 0 day(s). If the issue fee is paid on the date that is three months after the mailing date of this notice and the patent issues on the Tuesday before the date that is 28 weeks (six and a half months) after the mailing date of this notice, the Patent Term Adjustment will be 0 day(s).

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Application Information Retrieval (PAIR) WEB site (<http://pair.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

Notice of Allowability	Application No.		Applicant(s)	
	11/587,893		WRIGHT ET AL.	
	Examiner		Art Unit	
	David H. Kruse		1638	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to the Amendment filed 8 July 2010.
2. ☒ The allowed claim(s) is/are 1-51, 57-62 and 64-68.
3. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
 - a) ☐ All b) ☐ Some* c) ☐ None of the:
 1. ☐ Certified copies of the priority documents have been received.
 2. ☐ Certified copies of the priority documents have been received in Application No. ____.
 3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

* Certified copies not received: ____.

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.
THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.

4. ☐ A SUBSTITUTE OATH OR DECLARATION must be submitted. Note the attached EXAMINER'S AMENDMENT or NOTICE OF INFORMAL PATENT APPLICATION (PTO-152) which gives reason(s) why the oath or declaration is deficient.
5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.
 - (a) ☐ including changes required by the Notice of Draftsperson's Patent Drawing Review (PTO-948) attached
 - 1) ☐ hereto or 2) ☐ to Paper No./Mail Date ____.
 - (b) ☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date ____.

Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).
6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

Attachment(s)

1. ☐ Notice of References Cited (PTO-892) 2. ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948) 3. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date ____ 4. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material	5. ☐ Notice of Informal Patent Application 6. ☐ Interview Summary (PTO-413), Paper No./Mail Date ____ 7. ☐ Examiner's Amendment/Comment 8. ☐ Examiner's Statement of Reasons for Allowance 9. ☐ Other ____
--	--

/David H Kruse/ Primary Examiner, Art Unit 1638 17 September 2010	
---	--

Index of Claims 	Application/Control No. 11587893	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.
	Examiner David H Kruse	Art Unit 1638

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant
 ☐ CPA
 ☐ T.D.
 ☐ R.1.47

CLAIM		DATE									
Final	Original	09/17/2010									
1	1	=									
2	2	=									
3	3	=									
4	4	=									
5	5	=									
6	6	=									
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31	31	=									
32	32	=									
33	33	=									
34	34	=									
35	35	=									
36	36	=									

Index of Claims 	Application/Control No. 11587893	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.
	Examiner David H Kruse	Art Unit 1638

✓	Rejected	-	Cancelled	N	Non-Elected	A	Appeal
=	Allowed	÷	Restricted	I	Interference	O	Objected

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47									
CLAIM			DATE						
Final	Original	09/17/2010							
37	37	=							
38	38	=							
39	39	=							
40	40	=							
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57	62	=							
	63	-							
58	64	=							
59	65	=							
60	66	=							
61	67	=							
62	68	=							

Issue Classification 	Application/Control No. 11587893	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.
	Examiner David H Kruse	Art Unit 1638

ORIGINAL						INTERNATIONAL CLASSIFICATION												
CLASS			SUBCLASS			CLAIMED					NON-CLAIMED							
800			300			C	1	2	N	15 / 82 (2006.01.01)								
CROSS REFERENCE(S)						C	1	2	N	15 / 52 (2006.01.01)								
CLASS	SUBCLASS (ONE SUBCLASS PER BLOCK)																	
435	419																	
536	23.2																	
800	288	298																

☒ Claims renumbered in the same order as presented by applicant ☐ CPA ☐ T.D. ☐ R.1.47															
Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original	Final	Original

NONE		Total Claims Allowed:	
		62	
(Assistant Examiner) /David H Kruse/ Primary Examiner.Art Unit 1638	(Date) 09/17/2010	O.G. Print Claim(s) 1	O.G. Print Figure NONE
(Primary Examiner)	(Date)		

Search Notes 	Application/Control No. 11587893	Applicant(s)/Patent Under Reexamination WRIGHT ET AL.
	Examiner David H Kruse	Art Unit 1638

SEARCHED			
Class	Subclass	Date	Examiner
EAST	EAST	2/24/2010	/DK/

SEARCH NOTES		
Search Notes	Date	Examiner
Inventor Name Search	2/25/2010	/DK/
EAST: USPAT, USPGPUB, EPO, JPO, DERWENT	2/25/2010	/DK/
STN: AGRICOLA, BIOSIS, CAPLUS, EMBASE	2/26/2010	/DK/
Sequence Search SEQ ID NO: 9, included interference search.	12/17/2009	/DK/
Update EAST	9/17/2010	/DK/

INTERFERENCE SEARCH			
Class	Subclass	Date	Examiner
EAST	EAST	9/17/2010	/DK/

	/David H Kruse/ Primary Examiner.Art Unit 1638
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BIB DATA SHEET

CONFIRMATION NO. 7868

SERIAL NUMBER	FILING or 371(c) DATE	CLASS	GROUP ART UNIT	ATTORNEY DOCKET NO.
11/587,893	05/22/2008	800	1638	DAS-P0115-US-02
RULE				
APPLICANTS Terry R. Wright, Westfield, IN; Justin M. Lira, Fishers, IN; Donald J. Merlo, Carmel, IN; Nicole L. Arnold, Carmel, IN;				
** CONTINUING DATA ***** This application is a 371 of PCT/US2005/014737 05/02/2005 which claims benefit of 60/567,052 04/30/2004 /DK/				
** FOREIGN APPLICATIONS ***** NONE /DK/				
** IF REQUIRED, FOREIGN FILING LICENSE GRANTED ** 12/19/2008				
Foreign Priority claimed ☐ Yes ☒ No 35 USC 119(a-d) conditions met ☐ Yes ☒ No Verified and /DAVID H KRUSE/ Acknowledged Examiner's Signature		☐ Met after Allowance /DK/ Initials	STATE OR COUNTRY IN	SHEETS DRAWINGS 24
			TOTAL CLAIMS 67	INDEPENDENT CLAIMS 4
ADDRESS Baker & Daniels LLP- Dow AgroSciences 300 North Meridian Street, Suite 2700 Indianapolis, IN 46204 UNITED STATES				
TITLE Novel Herbicide Resistance Genes				
FILING FEE RECEIVED 3730	FEES: Authority has been given in Paper No. _____ to charge/credit DEPOSIT ACCOUNT No. _____ for following:			☐ All Fees ☐ 1.16 Fees (Filing) ☐ 1.17 Fees (Processing Ext. of time) ☐ 1.18 Fees (Issue) ☐ Other _____ ☐ Credit

EAST Search History**EAST Search History (Prior Art)**

Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	6	plant and tfda.clm.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2010/09/17 13:15
L2	3	(taurine adj dioxygenase) and plant. clm.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2010/09/17 13:15
S1	5	plant and tfda.clm.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2010/02/25 17:23
S2	28	aryloxyalkanoate adj dioxygenase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2010/02/25 17:31
S3	3	(taurine adj dioxygenase) and plant. clm.	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2010/02/26 14:34
S4	13	taurine adj dioxygenase	US-PGPUB; USPAT; USOCR; EPO; JPO; DERWENT	OR	ON	2010/02/26 14:41

EAST Search History (Interference)

< This search history is empty >

9/ 17/ 10 1:15:50 PM**C:\ Documents and Settings\ dkruse\ My Documents\ EAST\ Workspaces\ 11587893.wsp**

To: inteias@bakerd.com,cynthia.payson@bakerd.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 83067

Sep 24, 2010 06:05:54 AM

Dear PAIR Customer:

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UNITED STATES

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Application	Document	Mailroom Date	Attorney Docket No.
11587893	NOA	09/24/2010	DAS-P0115-US-02

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PATENT APPLICATION INFORMATION RETRIEVAL SYSTEM



PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), to: **Mail** Mail Stop ISSUE FEE
 Commissioner for Patents
 P.O. Box 1450
 Alexandria, Virginia 22313-1450
 or **Fax** (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence including the Patent, advance orders and notification of maintenance fees will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications.

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

83067 7590 09/24/2010

Baker & Daniels LLP- Dow AgroSciences
 300 North Meridian Street, Suite 2700
 Indianapolis, IN 46204

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being facsimile transmitted to the USPTO (571) 273-2885, on the date indicated below.

09/30/2010 SSANDAR1 00000003 041529 11587893

01 FC:1501 1510.00 DA
 02 FC:1504 300.00 DA

Michele Lyons (Depositor's name)
 [Signature] (Signature)
 September 29, 2010 (Date)

APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/587,893	05/22/2008	Terry R. Wright	DAS-P0115-US-02	7868

TITLE OF INVENTION: NOVEL HERBICIDE RESISTANCE GENES

APPLN. TYPE	SMALL ENTITY	ISSUE FEE DUE	PUBLICATION FEE DUE	PREV. PAID ISSUE FEE	TOTAL FEE(S) DUE	DATE DUE
nonprovisional	NO	\$1510	\$300	\$0	\$1810	12/27/2010

EXAMINER	ART UNIT	CLASS-SUBCLASS
KRUSE, DAVID H	1638	800-300000

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).

- ☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/SB/47; Rev 03-02 or more recent) attached. Use of a Customer Number is required.

2. For printing on the patent front page, list

- (1) the names of up to 3 registered patent attorneys or agents OR, alternatively,
 (2) the name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

- 1 Kenneth B. Ludwig
 2 BAKER & DANIELS LLP
 3 _____

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document has been filed for recordation as set forth in 37 CFR 3.11. Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE

Dow AgroSciences LLC

(B) RESIDENCE: (CITY and STATE OR COUNTRY)

Indianapolis IN USA

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. The following fee(s) are submitted:

- ☒ Issue Fee
☒ Publication Fee (No small entity discount permitted)
☐ Advance Order - # of Copies _____

4b. Payment of Fee(s): (Please first reapply any previously paid issue fee shown above)

- ☐ A check is enclosed.
☒ Payment by credit card. Form PTO-2038 is attached.
☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment, to Deposit Account Number _____ (enclose an extra copy of this form).

5. Change in Entity Status (from status indicated above)

- ☐ a. Applicant claims SMALL ENTITY status. See 37 CFR 1.27. ☐ b. Applicant is no longer claiming SMALL ENTITY status. See 37 CFR 1.27(g)(2).

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Authorized Signature

Kenneth B. Ludwig

Date

9-29-2010

Typed or printed name

Kenneth B. Ludwig

Registration No.

42,814

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APPLICATION NO.	ISSUE DATE	PATENT NO.	ATTORNEY DOCKET NO.	CONFIRMATION NO.
11/587,893	11/23/2010	7838733	DAS-P0115-US-02	7868

83067 7590 11/03/2010

Baker & Daniels LLP- Dow AgroSciences
300 North Meridian Street, Suite 2700
Indianapolis, IN 46204

ISSUE NOTIFICATION

The projected patent number and issue date are specified above.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b) (application filed on or after May 29, 2000)

The Patent Term Adjustment is 360 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

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APPLICANT(s) (Please see PAIR WEB site <http://pair.uspto.gov> for additional applicants):

Terry R. Wright, Westfield, IN;
Justin M. Lira, Fishers, IN;
Donald J. Merlo, Carmel, IN;
Nicole L. Arnold, Carmel, IN;

To: inteas@bakerd.com,cynthia.payson@bakerd.com,
From: PAIR_eOfficeAction@uspto.gov
Cc: PAIR_eOfficeAction@uspto.gov
Subject: Private PAIR Correspondence Notification for Customer Number 83067

Nov 04, 2010 05:36:19 AM

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Application	Document	Mailroom Date	Attorney Docket No.
11587893	ISSUE.NTF	11/03/2010	DAS-P0115-US-02

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