US 20240074374A1

(19) United States

(12) Patent Application Publication (10) Pub. No.: US 2024/0074374 A1

Bernacchi et al. (43) Pub. Date: Mar. 7, 2024

(54) EFFECTS OF A PLURALITY OF MUTATIONS TO IMPROVE HERBICIDE RESISTANCE/TOLERANCE IN RICE

(71) Applicant: **RiceTec, Inc.**, Alvin, TX (US)

(72) Inventors: **Dario Bernacchi**, Friendswood, TX (US); **Caleb Knepper**, League City, TX (US)

(21) Appl. No.: **18/457,067**

(22) Filed: **Aug. 28, 2023**

Related U.S. Application Data

(63) Continuation of application No. 15/863,559, filed on Jan. 5, 2018.

(60) Provisional application No. 62/573,451, filed on Oct. 17, 2017, provisional application No. 62/508,264, filed on May 18, 2017, provisional application No. 62/453,094, filed on Feb. 1, 2017, provisional application No. 62/452,800, filed on Jan. 31, 2017.

Publication Classification

(51) **Int. Cl.**

A01H 6/46	(2006.01)
A01H 5/10	(2006.01)
A01N 43/50	(2006.01)
C12N 15/82	(2006.01)

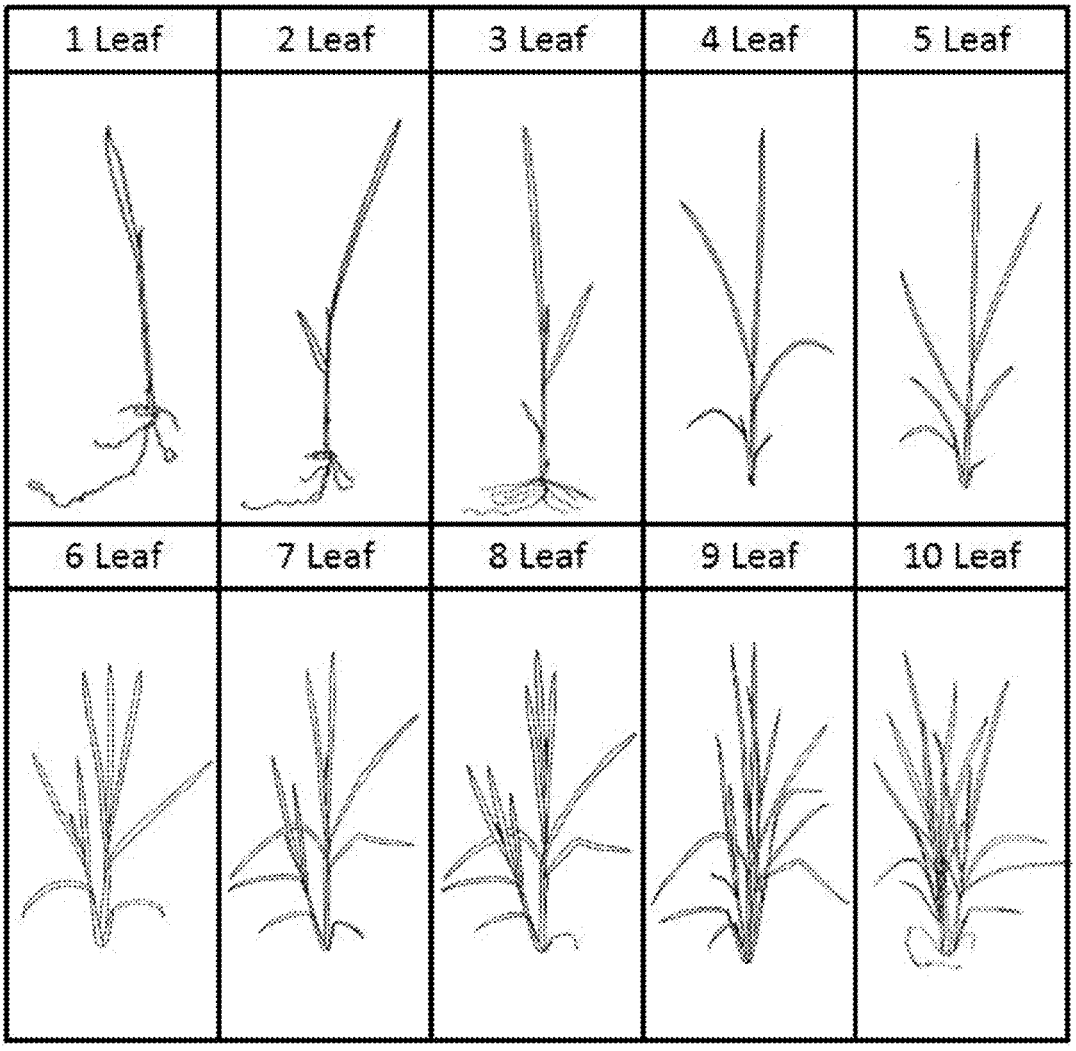
(52) **U.S. Cl.**

CPC ***A01H 6/4636*** (2018.05); ***A01H 5/10*** (2013.01); ***A01N 43/50*** (2013.01); ***C12N 15/8274*** (2013.01)

(57) **ABSTRACT**

Rice is described that is tolerant/resistant to AHAS/ALS inhibitors because of a plurality of mutations that act synergistically in providing resistance/tolerance to the herbicide. Tolerance/resistance is due to presence of combined mutations in the rice leading to amino acid substitutions (A205V and G654E) in the AHAS/ALS enzyme. Use of the rice for weed control and methods of producing tolerant/resistant rice are also disclosed.

Specification includes a Sequence Listing.



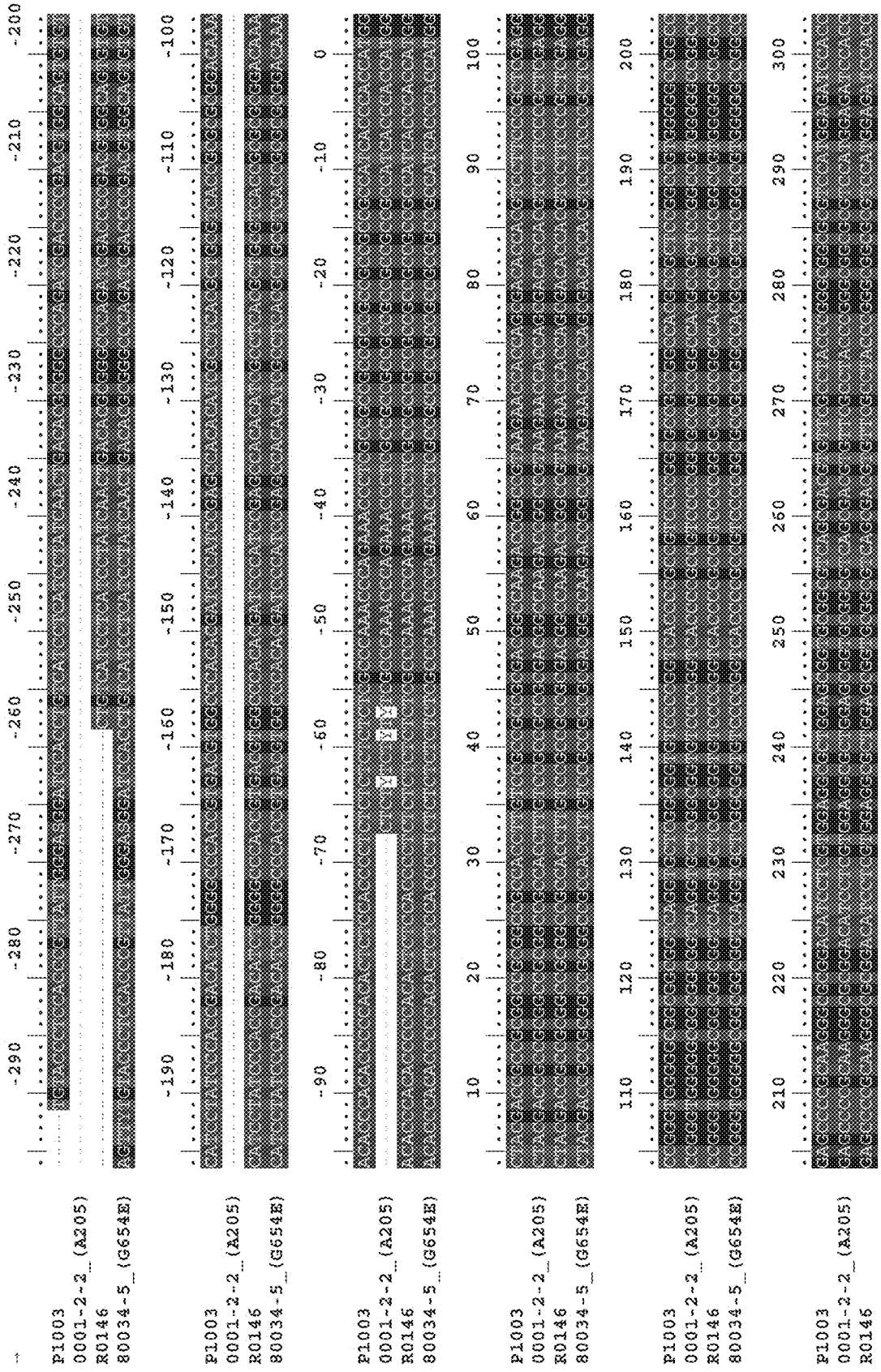


FIG. 1

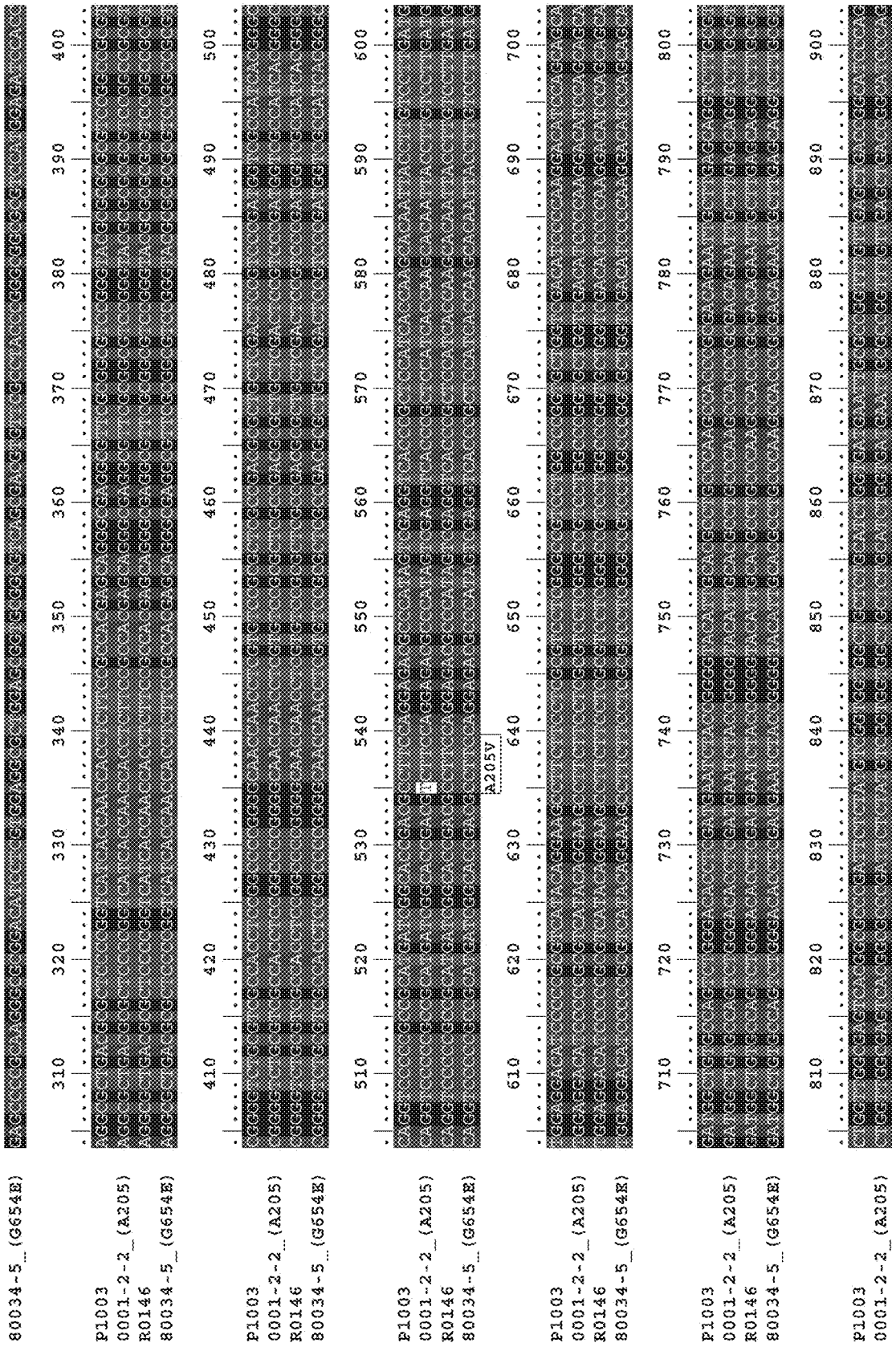


FIG. 1 (cont.)

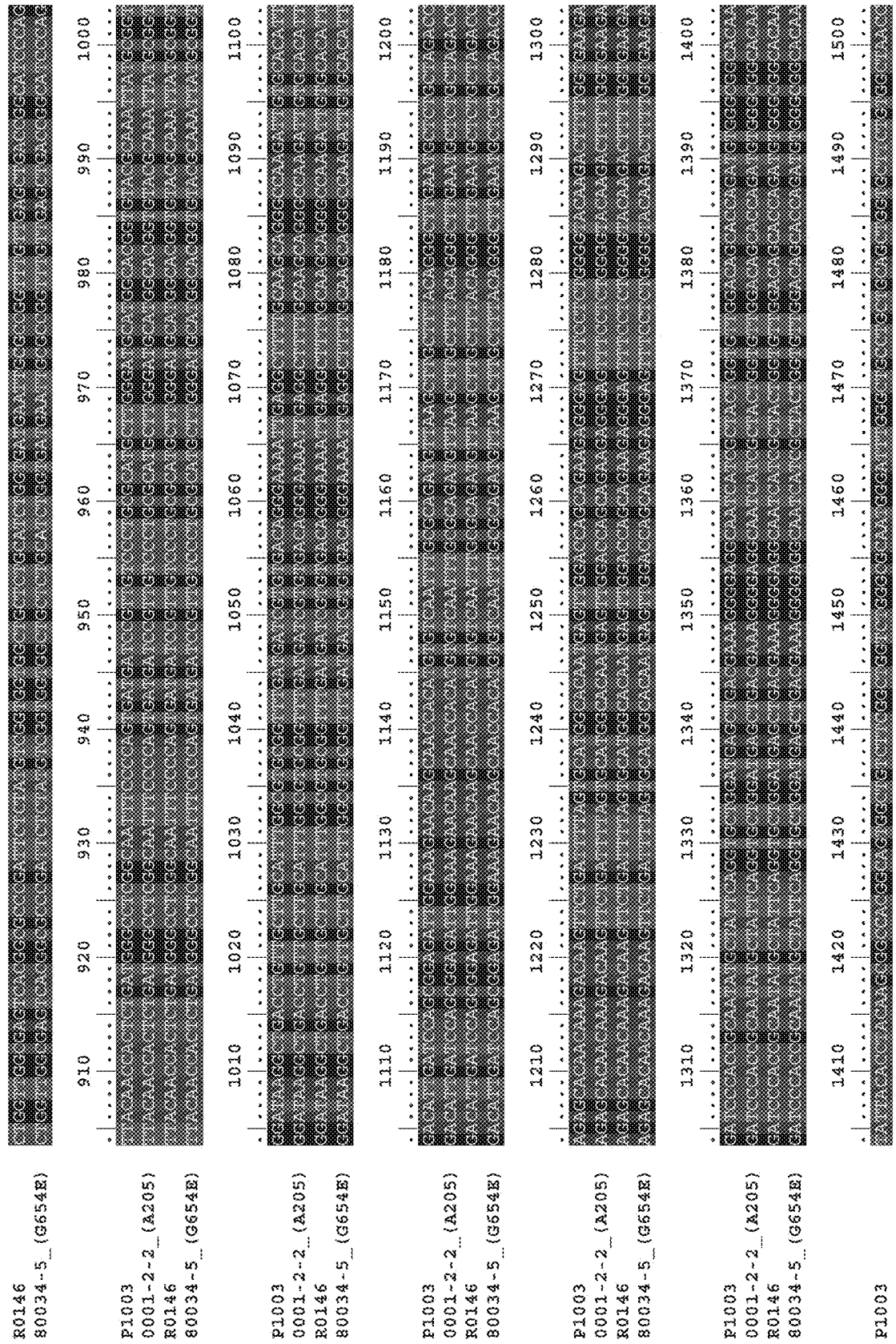


FIG. 1 (cont.)

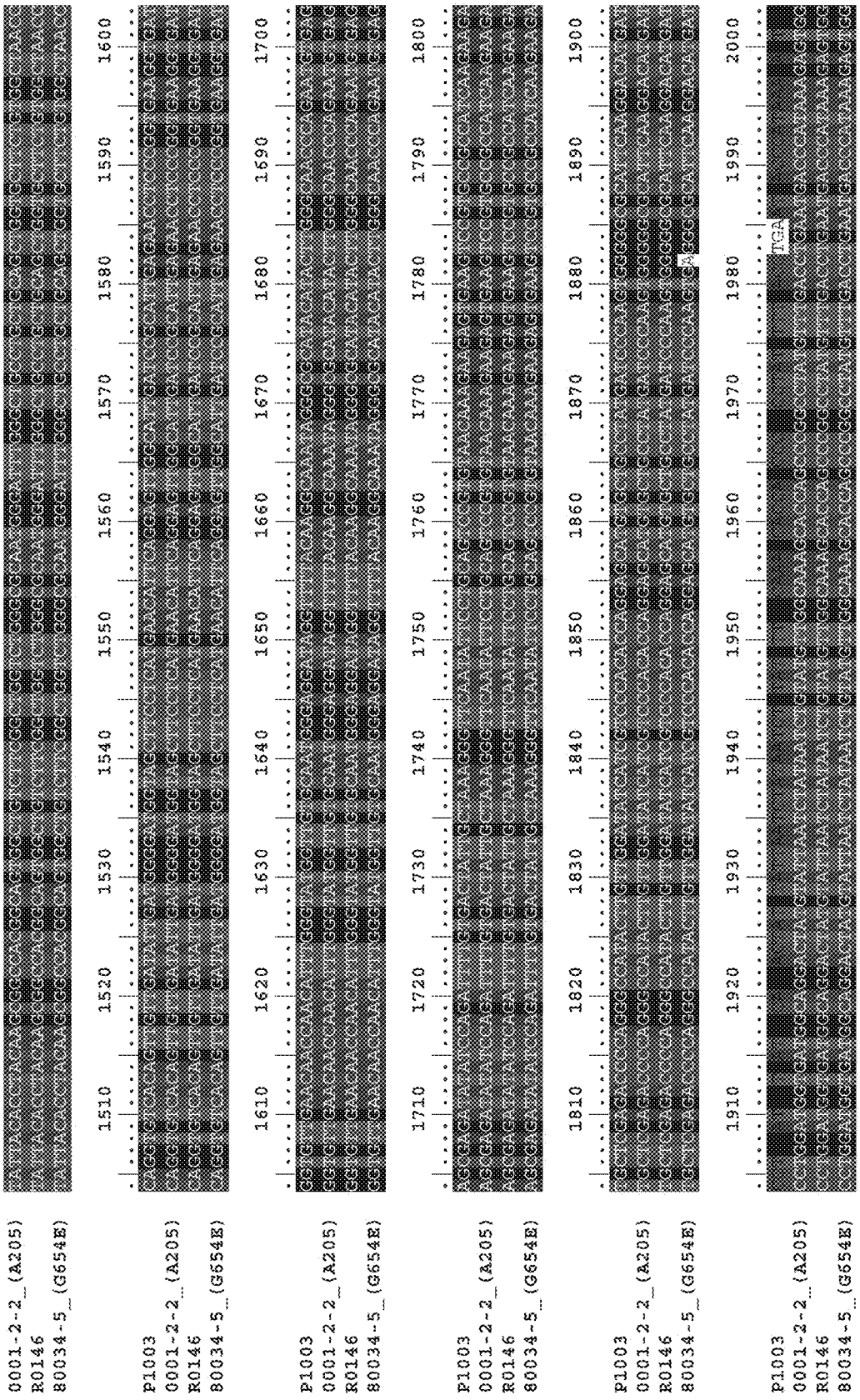

0001-2-2_(A205)
R0146
80034-5_(G654E)
P1003
1510
1520
1530
1540
1550
1560
1570
1580
1590
1600
1610
1620
1630
1640
1650
1660
1670
1680
1690
1700
1710
1720
1730
1740
1750
1760
1770
1780
1790
1800
1810
1820
1830
1840
1850
1860
1870
1880
1890
1900
1910
1920
1930
1940
1950
1960
1970
1980
1990
2000
TGA

FIG. 1 (cont.)

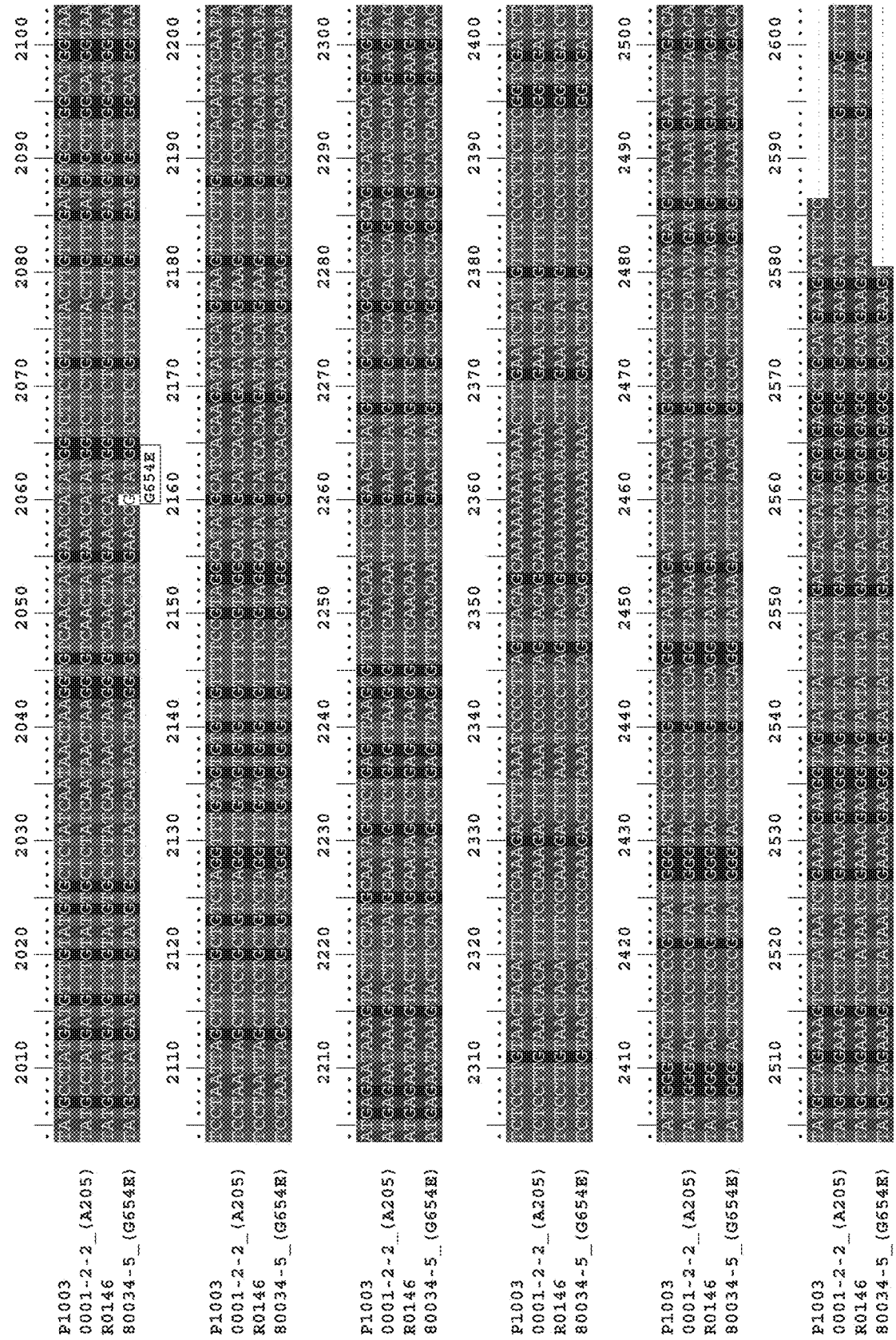


FIG. 1 (cont.)

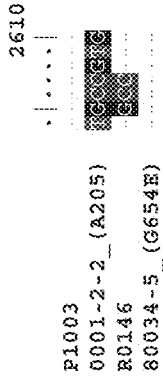


FIG. 1 (cont.)

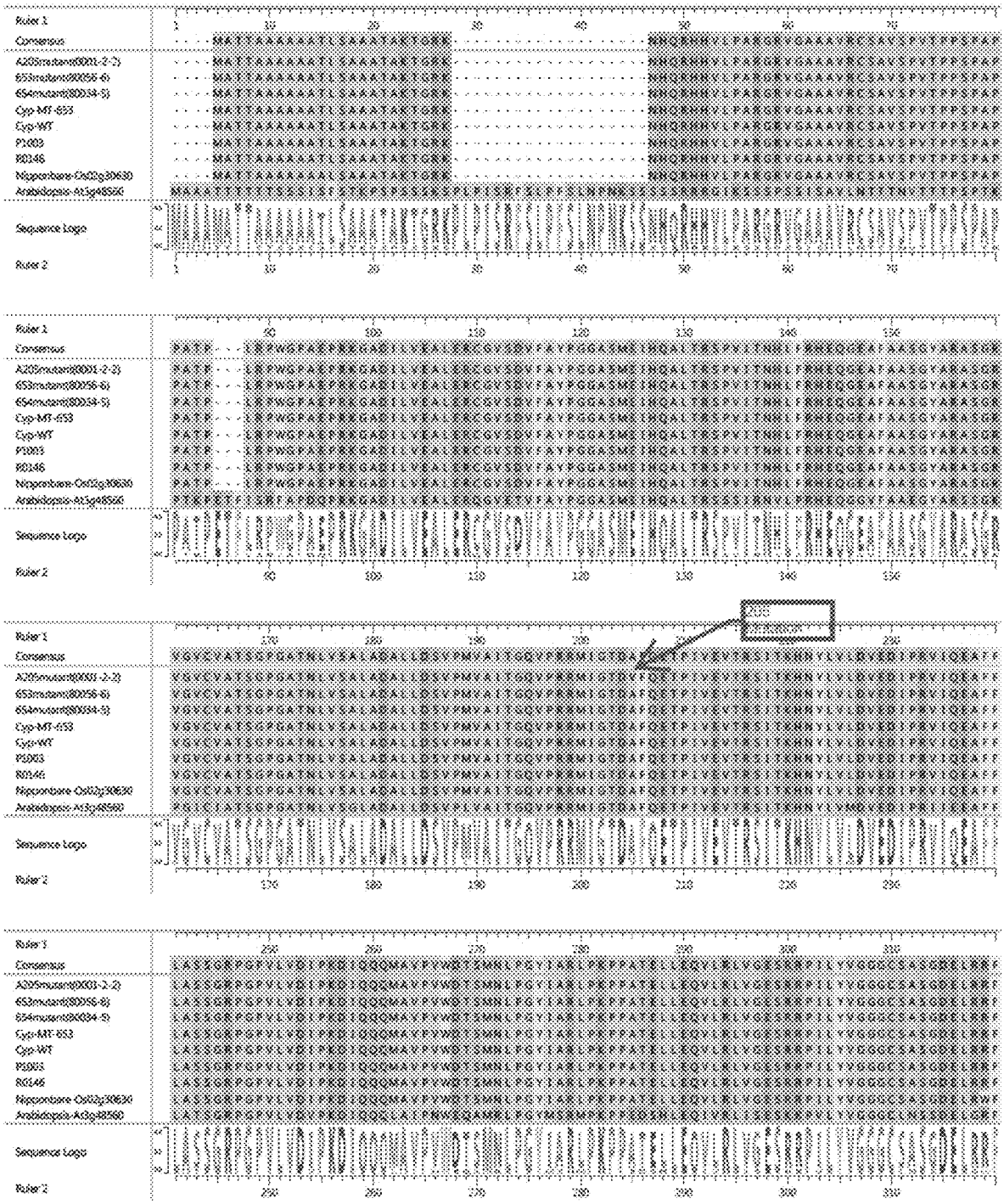

Ruler 1
Consensus
A205mutant(80001-2-2)
653mutant(80056-8)
654mutant(80034-5)
Cyp-MT-653
Cyp-WT
P1003
R0146
Nipponbare-Os02g30630
Arabidopsis-At3g48560
Sequence Logo
Ruler 2

FIG. 2

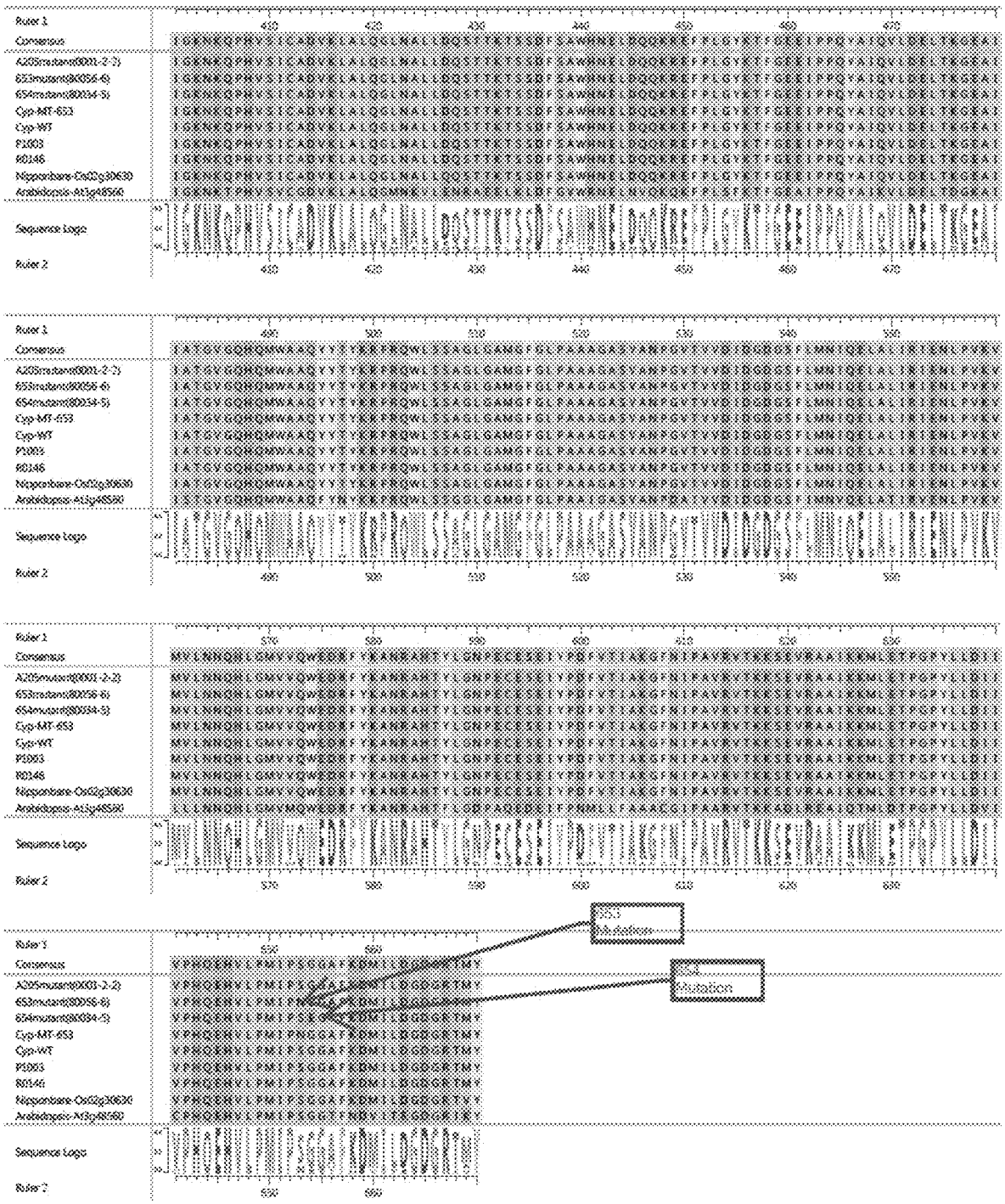

Ruler 1
Consensus
A205mutant(6001-2-2)
653mutant(80056-6)
654mutant(80034-5)
Cyp-MT-653
Cyp-WT
P1003
80146
Nipponbare-Os02g30630
Arabidopsis-At3g48560
Sequence Logo
Ruler 2
P450 N-Terminus
Mutation

FIG. 2 (cont.)

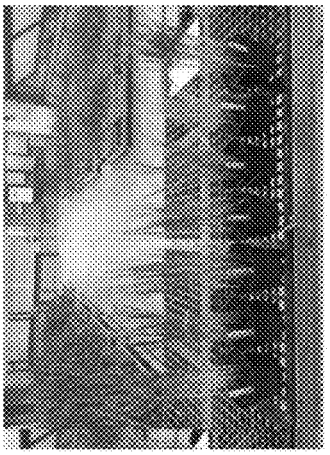


FIG. 3C

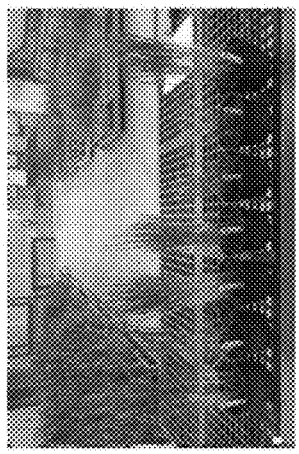


FIG. 3F

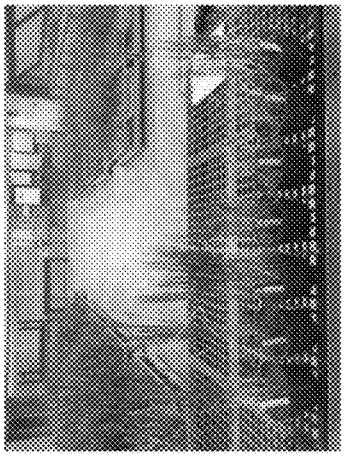


FIG. 3I

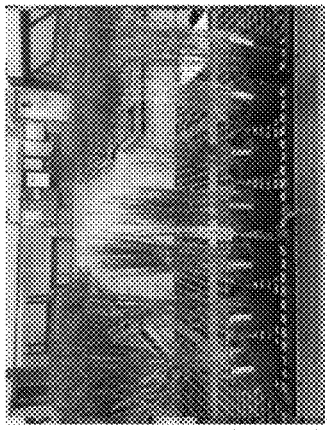


FIG. 3B

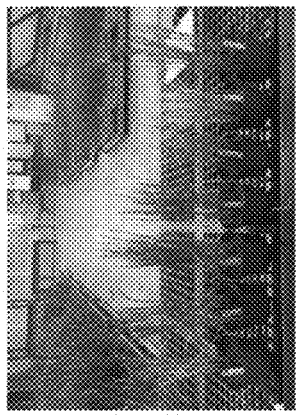


FIG. 3E

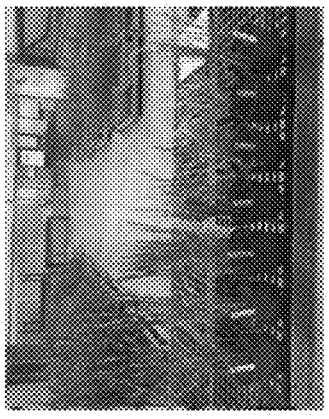


FIG. 3H

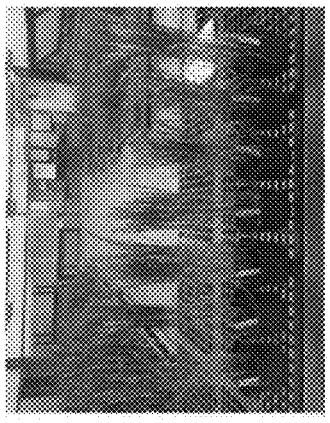


FIG. 3A

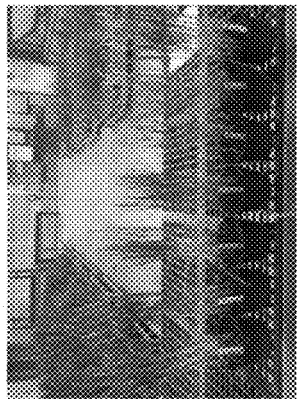


FIG. 3D

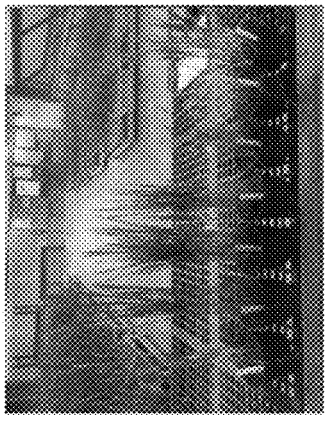


FIG. 3G

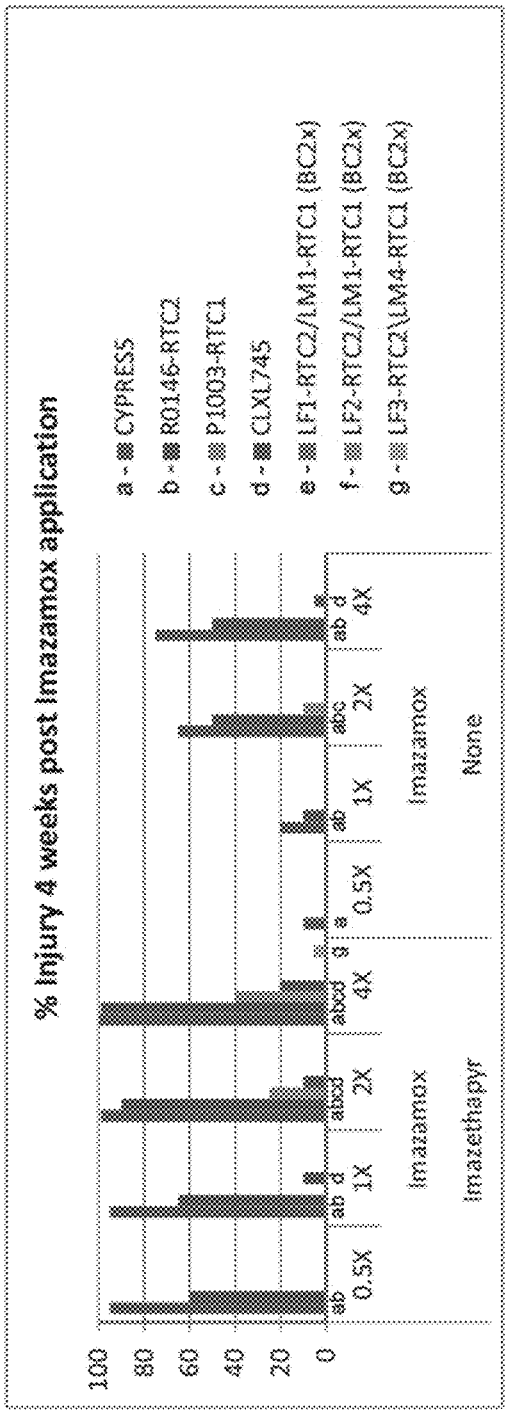

% Injury 4 weeks post Imazamox application
a - CYPRESS
b - R0146-RTC2
c - P1003-RTC1
d - CLXL745
e - LF1-RTC2/LM1-RTC1 (BC2x)
f - LF2-RTC2/LM1-RTC1 (BC2x)
g - LF3-RTC2\LM4-RTC1 (BC2x)
100
80
60
40
20
0
ab
a
ab d
abcd
abcd
g
g
a
ab
abc
ab d
a
0.5X
1X
2X
4X
0.5X
1X
2X
4X
Imazamox
Imazamox
None
Imazethapyr

FIG. 4

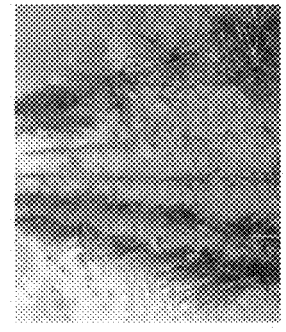


FIG. 5D

FIG. 5C

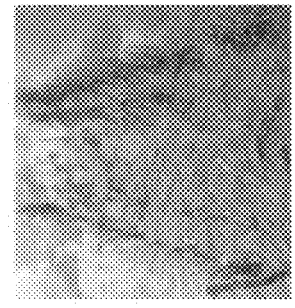


FIG. 5B

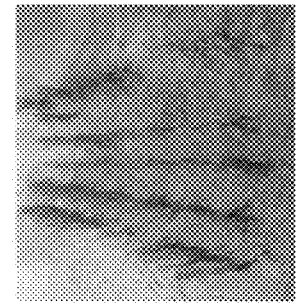


FIG. 5A

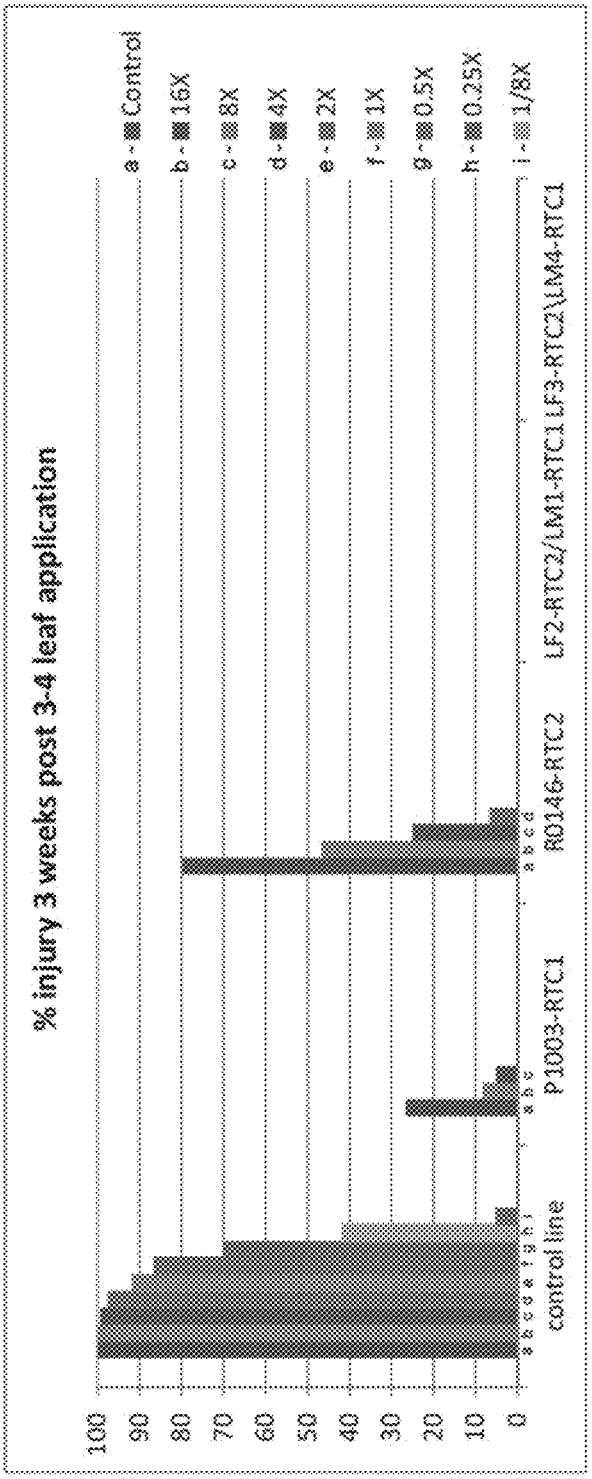

% injury 3 weeks post 3-4 leaf application
100
90
80
70
60
50
40
30
20
10
0
a
a
a
a
a
a
a
a
a
b
c
d
a
b
c
d
a
b
c
d
control line
P1003-RTC1
R0146-RTC2
LF2-RTC2/LM1-RTC1
LF3-RTC2\LM4-RTC1
a - Control
b - 16X
c - 8X
d - 4X
e - 2X
f - 1X
g - 0.5X
h - 0.25X
i - 1/8X

FIG. 6

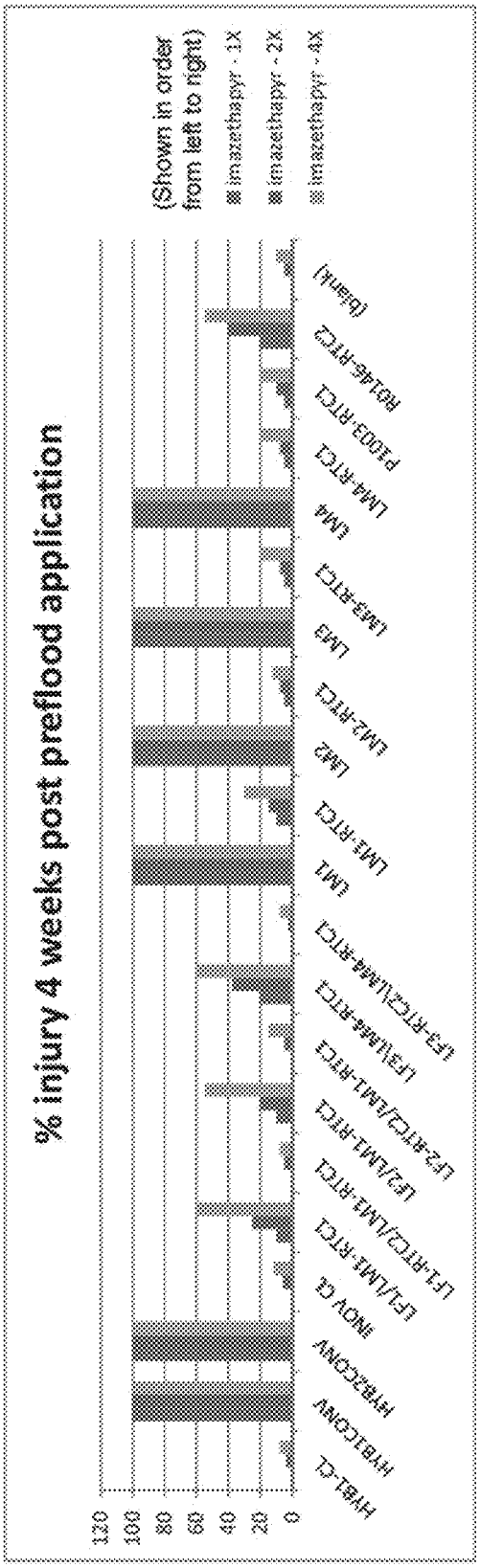

% injury 4 weeks post preflood application
(Shown in order from left to right)
imazethapyr - 1X
imazethapyr - 2X
imazethapyr - 4X
0
20
40
60
80
100
120
HVB1-CL
HVB1CONV
HVB2CONV
ENOV CL
LF1/LM1-RTC1
LF1-RTC2/LM1-RTC1
LF2/LM1-RTC1
LF2-RTC2/LM1-RTC1
LF3/LM4-RTC1
LF3-RTC2/LM4-RTC1
LM1
LM1-RTC1
LM2
LM2-RTC1
LM3
LM3-RTC1
LM4
LM4-RTC1
P1003-RTC1
R0146-RTC2
(blank)

FIG. 7

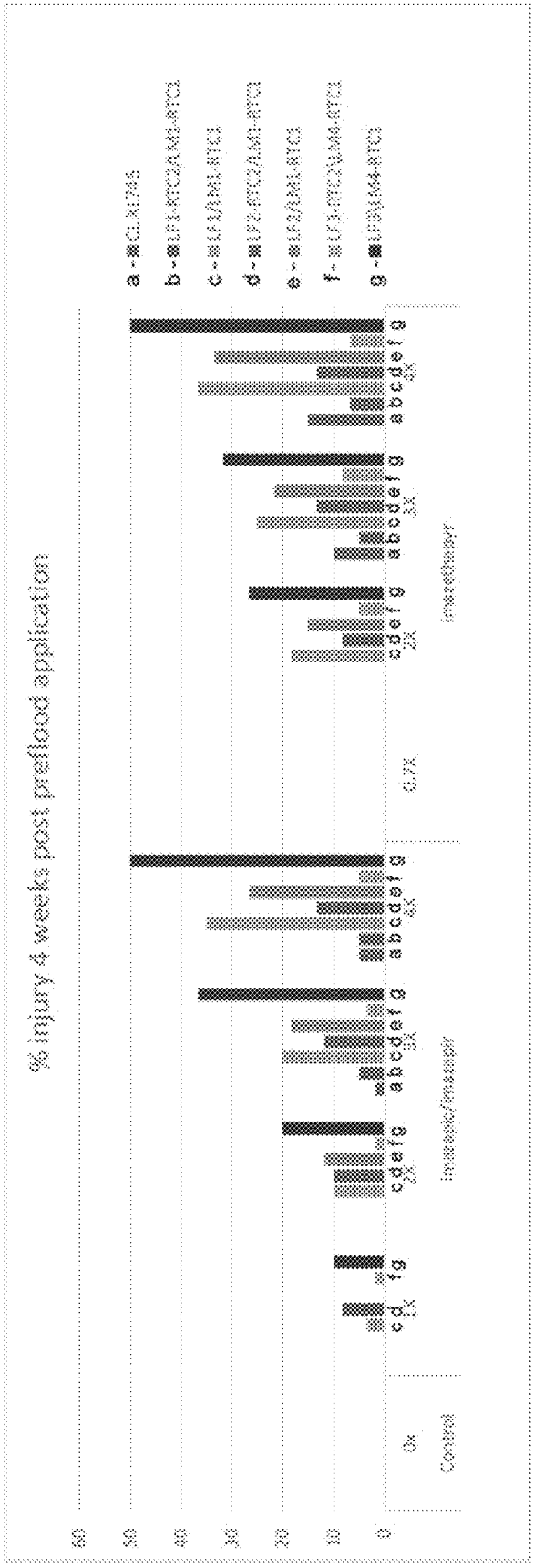

% injury 4 weeks post preflood application
60
50
40
30
20
10
0
Control
0x
1x
2x
3x
4x
5x
6x
7x
8x
9x
10x
11x
12x
13x
14x
15x
16x
17x
18x
19x
20x
21x
22x
23x
24x
25x
26x
27x
28x
29x
30x
31x
32x
33x
34x
35x
36x
37x
38x
39x
40x
41x
42x
43x
44x
45x
46x
47x
48x
49x
50x
51x
52x
53x
54x
55x
56x
57x
58x
59x
60x
61x
62x
63x
64x
65x
66x
67x
68x
69x
70x
71x
72x
73x
74x
75x
76x
77x
78x
79x
80x
81x
82x
83x
84x
85x
86x
87x
88x
89x
90x
91x
92x
93x
94x
95x
96x
97x
98x
99x
100x
101x
102x
103x
104x
105x
106x
107x
108x
109x
110x
111x
112x
113x
114x
115x
116x
117x
118x
119x
120x
121x
122x
123x
124x
125x
126x
127x
128x
129x
130x
131x
132x
133x
134x
135x
136x
137x
138x
139x
140x
141x
142x
143x
144x
145x
146x
147x
148x
149x
150x
151x
152x
153x
154x
155x
156x
157x
158x
159x
160x
161x
162x
163x
164x
165x
166x
167x
168x
169x
170x
171x
172x
173x
174x
175x
176x
177x
178x
179x
180x
181x
182x
183x
184x
185x
186x
187x
188x
189x
190x
191x
192x
193x
194x
195x
196x
197x
198x
199x
200x
201x
202x
203x
204x
205x
206x
207x
208x
209x
210x
211x
212x
213x
214x
215x
216x
217x
218x
219x
220x
221x
222x
223x
224x
225x
226x
227x
228x
229x
230x
231x
232x
233x
234x
235x
236x
237x
238x
239x
240x
241x
242x
243x
244x
245x
246x
247x
248x
249x
250x
251x
252x
253x
254x
255x
256x
257x
258x
259x
260x
261x
262x
263x
264x
265x
266x
267x
268x
269x
270x
271x
272x
273x
274x
275x
276x
277x
278x
279x
280x
281x
282x
283x
284x
285x
286x
287x
288x
289x
290x
291x
292x
293x
294x
295x
296x
297x
298x
299x
300x
301x
302x
303x
304x
305x
306x
307x
308x
309x
310x
311x
312x
313x
314x
315x
316x
317x
318x
319x
320x
321x
322x
323x
324x
325x
326x
327x
328x
329x
330x
331x
332x
333x
334x
335x
336x
337x
338x
339x
340x
341x
342x
343x
344x
345x
346x
347x
348x
349x
350x
351x
352x
353x
354x
355x
356x
357x
358x
359x
360x
361x
362x
363x
364x
365x
366x
367x
368x
369x
370x
371x
372x
373x
374x
375x
376x
377x
378x
379x
380x
381x
382x
383x
384x
385x
386x
387x
388x
389x
390x
391x
392x
393x
394x
395x
396x
397x
398x
399x
400x
401x
402x
403x
404x
405x
406x
407x
408x
409x
410x
411x
412x
413x
414x
415x
416x
417x
418x
419x
420x
421x
422x
423x
424x
425x
426x
427x
428x
429x
430x
431x
432x
433x
434x
435x
436x
437x
438x
439x
440x
441x
442x
443x
444x
445x
446x
447x
448x
449x
450x
451x
452x
453x
454x
455x
456x
457x
458x
459x
460x
461x
462x
463x
464x
465x
466x
467x
468x
469x
470x
471x
472x
473x
474x
475x
476x
477x
478x
479x
480x
481x
482x
483x
484x
485x
486x
487x
488x
489x
490x
491x
492x
493x
494x
495x
496x
497x
498x
499x
500x
501x
502x
503x
504x
505x
506x
507x
508x
509x
510x
511x
512x
513x
514x
515x
516x
517x
518x
519x
520x
521x
522x
523x
524x
525x
526x
527x
528x
529x
530x
531x
532x
533x
534x
535x
536x
537x
538x
539x
540x
541x
542x
543x
544x
545x
546x
547x
548x
549x
550x
551x
552x
553x
554x
555x
556x
557x
558x
559x
560x
561x
562x
563x
564x
565x
566x
567x
568x
569x
570x
571x
572x
573x
574x
575x
576x
577x
578x
579x
580x
581x
582x
583x
584x
585x
586x
587x
588x
589x
590x
591x
592x
593x
594x
595x
596x
597x
598x
599x
600x
601x
602x
603x
604x
605x
606x
607x
608x
609x
610x
611x
612x
613x
614x
615x
616x
617x
618x
619x
620x
621x
622x
623x
624x
625x
626x
627x
628x
629x
630x
631x
632x
633x
634x
635x
636x
637x
638x
639x
640x
641x
642x
643x
644x
645x
646x
647x
648x
649x
650x
651x
652x
653x
654x
655x
656x
657x
658x
659x
660x
661x
662x
663x
664x
665x
666x
667x
668x
669x
670x
671x
672x
673x
674x
675x
676x
677x
678x
679x
680x
681x
682x
683x
684x
685x
686x
687x
688x
689x
690x
691x
692x
693x
694x
695x
696x
697x
698x
699x
700x
701x
702x
703x
704x
705x
706x
707x
708x
709x
710x
711x
712x
713x
714x
715x
716x
717x
718x
719x
720x
721x
722x
723x
724x
725x
726x
727x
728x
729x
730x
731x
732x
733x
734x
735x
736x
737x
738x
739x
740x
741x
742x
743x
744x
745x
746x
747x
748x
749x
750x
751x
752x
753x
754x
755x
756x
757x
758x
759x
760x
761x
762x
763x
764x
765x
766x
767x
768x
769x
770x
771x
772x
773x
774x
775x
776x
777x
778x
779x
780x
781x
782x
783x
784x
785x
786x
787x
788x
789x
790x
791x
792x
793x
794x
795x
796x
797x
798x
799x
800x
801x
802x
803x
804x
805x
806x
807x
808x
809x
810x
811x
812x
813x
814x
815x
816x
817x
818x
819x
820x
821x
822x
823x
824x
825x
826x
827x
828x
829x
830x
831x
832x
833x
834x
835x
836x
837x
838x
839x
840x
841x
842x
843x
844x
845x
846x
847x
848x
849x
850x
851x
852x
853x
854x
855x
856x
857x
858x
859x
860x
861x
862x
863x
864x
865x
866x
867x
868x
869x
870x
871x
872x
873x
874x
875x
876x
877x
878x
879x
880x
881x
882x
883x
884x
885x
886x
887x
888x
889x
890x
891x
892x
893x
894x
895x
896x
897x
898x
899x
900x
901x
902x
903x
904x
905x
906x
907x
908x
909x
910x
911x
912x
913x
914x
915x
916x
917x
918x
919x
920x
921x
922x
923x
924x
925x
926x
927x
928x
929x
930x
931x
932x
933x
934x
935x
936x
937x
938x
939x
940x
941x
942x
943x
944x
945x
946x
947x
948x
949x
950x
951x
952x
953x
954x
955x
956x
957x
958x
959x
960x
961x
962x
963x
964x
965x
966x
967x
968x
969x
970x
971x
972x
973x
974x
975x
976x
977x
978x
979x
980x
981x
982x
983x
984x
985x
986x
987x
988x
989x
990x
991x
992x
993x
994x
995x
996x
997x
998x
999x
1000x

FIG. 8

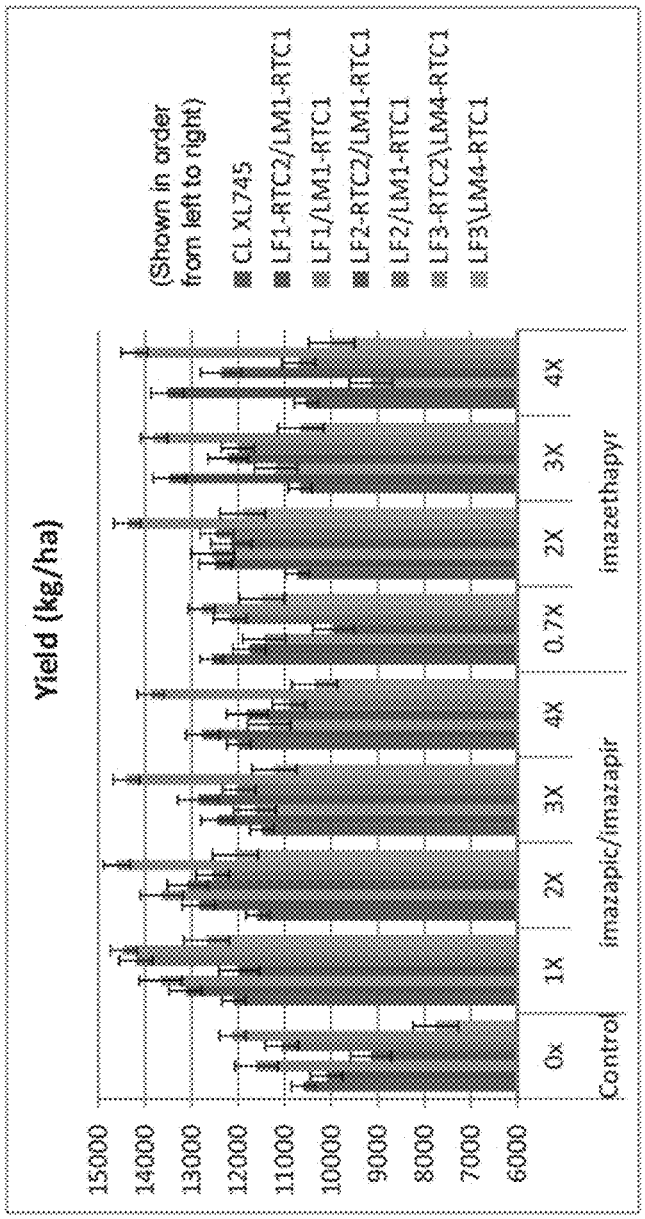

Yield (kg/ha)
15000
14000
13000
12000
11000
10000
9000
8000
7000
6000
Control
0x
1X
2X
3X
4X
imazapic/imazapir
0.7X
2X
3X
4X
imazethapyr
(Shown in order from left to right)
CL XL745
LF1-RTC2/LM1-RTC1
LF1/LM1-RTC1
LF2-RTC2/LM1-RTC1
LF2/LM1-RTC1
LF3-RTC2\LM4-RTC1
LF3\LM4-RTC1

FIG. 9

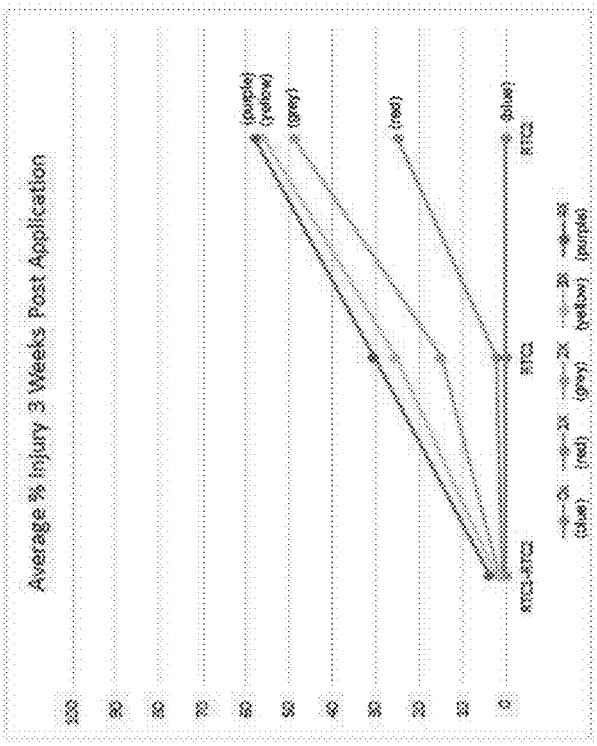


FIG. 10B

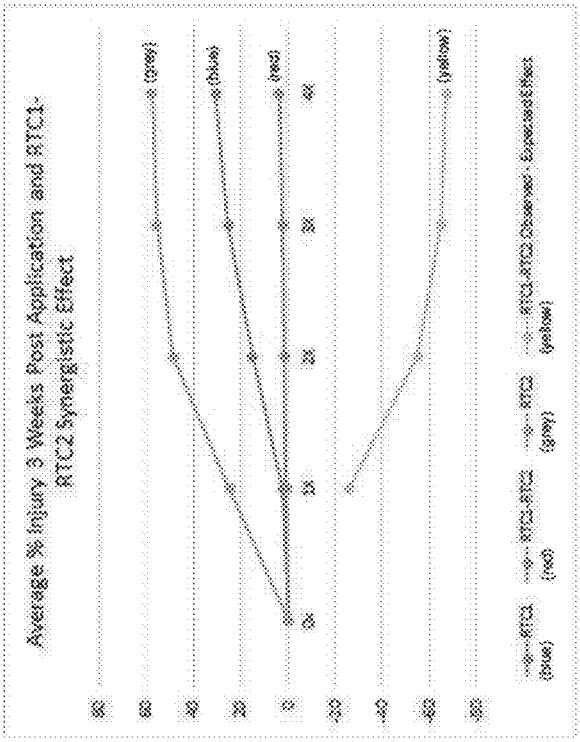


FIG. 10A

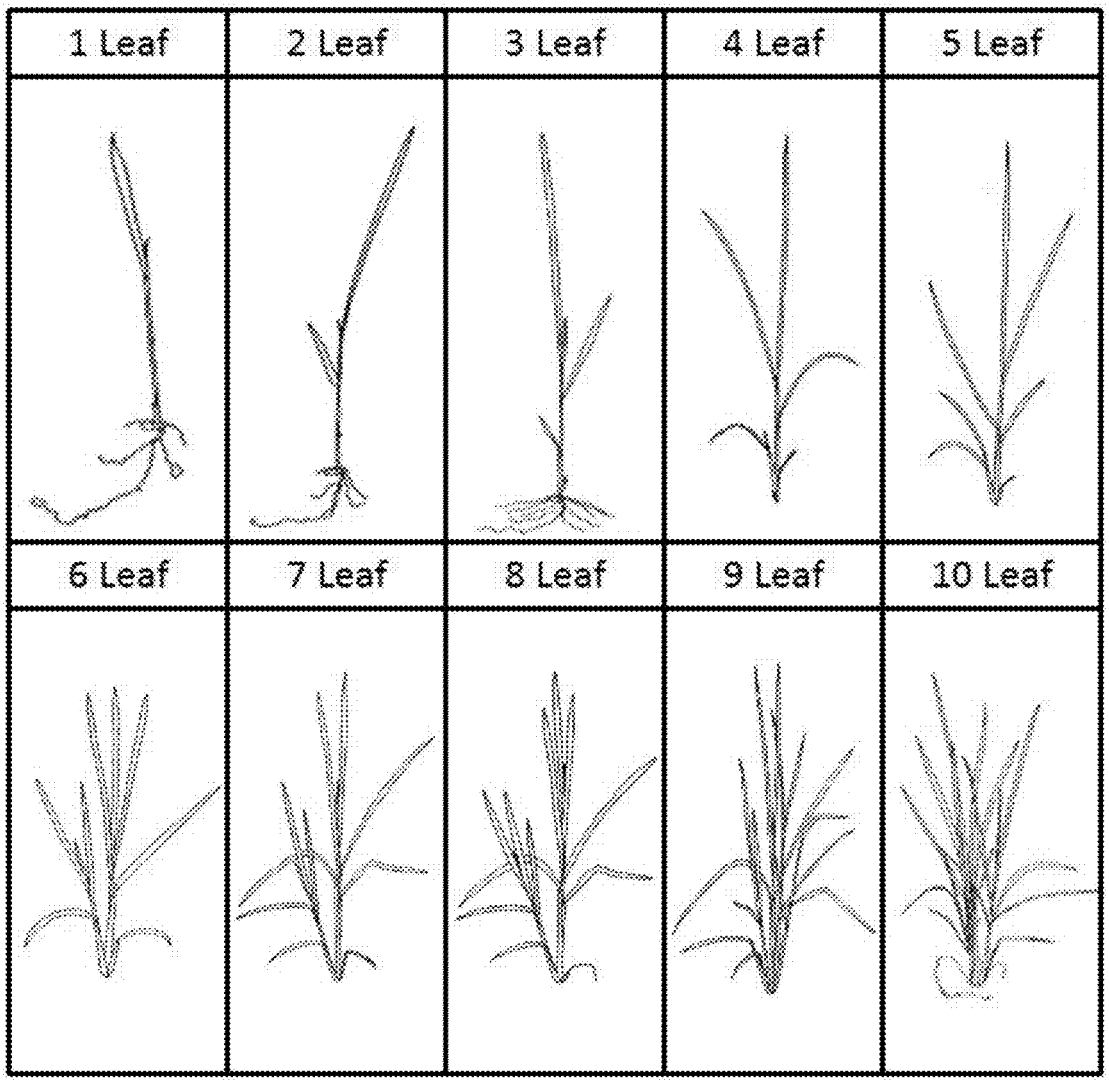

1 Leaf
2 Leaf
3 Leaf
4 Leaf
5 Leaf
6 Leaf
7 Leaf
8 Leaf
9 Leaf
10 Leaf

FIG. 11

EFFECTS OF A PLURALITY OF MUTATIONS TO IMPROVE HERBICIDE RESISTANCE/TOLERANCE IN RICE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] The application is a continuation of U.S. patent application Ser. No. 15/863,559, filed Jan. 5, 2018, which claims the benefit of priority under 35 U.S.C. § 119(e) to U.S. Provisional Application No. 62/452,800, filed Jan. 31, 2017, U.S. Provisional Application No. 62/453,094, filed Feb. 1, 2017, U.S. Provisional Application No. 62/508,264, filed May 18, 2017, and U.S. Provisional Application No. 62/573,451, filed Oct. 17, 2017. The disclosures set forth in the referenced applications are incorporated herein by reference in their entireties.

BACKGROUND

Sequence Listing

[0002] The instant application contains a Sequence Listing which has been submitted electronically in HTML format and is hereby incorporated by reference in its entirety. Said HTML copy, created on Aug. 25, 2023, is named 01215_SEQ_ST.26.xml and is 29,087 bytes in size.

[0003] Mutant rice is disclosed that is (1) resistant/tolerant to AHAS/ALS inhibitors especially imidazolinone ("IMI") herbicides, at a relatively high concentration of inhibitors, and (2) wherein the mutations effect synergistic responses in rice to the herbicides. Methods of weed control are disclosed using herbicide resistant/tolerant rice with these mutations as crops in fields. Methods to produce herbicide resistant/tolerant rice are also disclosed.

Improving Value of Rice Crops

[0004] Rice is an ancient agricultural crop and today is one of the principal food crops of the world. There are two cultivated species of rice: *Oryza sativa L.,* the Asian rice, and *Oryza glaberrima Steud,* the African rice. The Asian species constitutes virtually all of the world's cultivated rice and is the species grown in the United States. Three major rice producing regions exist in the United States: the Mississippi Delta (Arkansas, Mississippi, northeast Louisiana, southeast Missouri), the Gulf Coast (southwest Louisiana, southeast Texas), and the Central Valley of California. Other countries, in particular in South America and the East, are major rice producers.

[0005] Rice is one of the few crops that can be grown in a shallow flood as it has a unique structure allowing gas exchange through the stems between the roots and the atmosphere. Growth in a shallow flood results in the best yields and is the reason that rice is usually gown in heavy clay soils, or soils with an impermeable hard pan layer just below the soil surface. These soil types are usually either not suitable for other crops or at best, the crops yield poorly.

[0006] The constant improvement of rice is imperative to provide necessary nutrition for a growing world population. A large portion of the world population consumes rice as their primary source of nutrition, and crops must thrive in various environmental conditions including competing with weeds and attacks by unfavorable agents. Rice improvement is carried out through conventional breeding practices and also by recombinant genetic techniques. Though appearing straightforward to those outside this discipline, crop improvement requires keen scientific and artistic skill and results are generally unpredictable.

[0007] Although specific breeding objectives vary somewhat in the different rice producing regions of the world, increasing yield is a primary objective in all programs.

[0008] Plant breeding begins with the analysis and definition of strengths and weaknesses of cultivars in existence, followed by the establishment of program goals to improve areas of weakness to produce new cultivars. Specific breeding objectives include combining in a single cultivar an improved combination of desirable traits from the parental sources. Desirable traits may be introduced due to spontaneous or induced mutations. Desirable traits include higher yield, resistance to environmental stress, diseases, and insects, better stems and roots, tolerance to low temperatures, better agronomic characteristics, nutritional value and grain quality.

[0009] For example, the breeder initially selects and crosses two or more parental lines, followed by selection for desired traits among the many new genetic combinations. The breeder can theoretically generate billions of new and different genetic combinations via crossing. Breeding by using crossing and selfing, does not imply direct control at the cellular level. However, that type of control may be achieved in part using recombinant genetic techniques.

[0010] Pedigree breeding is used commonly for the improvement of self-pollinating crops such as rice. For example, two parents which possess favorable, complementary traits are crossed to produce an F1 generation. One or both parents may themselves represent an F1 from a previous cross. Subsequently a segregating population is produced, by growing the seeds resulting from selfing one or several F1s if the two parents are pure lines, or by directly growing the seed resulting from the initial cross if at least one of the parents is an F1. Selection of the best individual genomes may begin in the first segregating population or F2; then, beginning in the F3, the best individuals in the best families are selected. "Best" is defined according to the goals of a particular breeding program e.g., to increase yield, resist diseases. Overall a multifactorial approach is used to define "best" because of genetic interactions. A desirable gene in one genetic background may differ in a different background. In addition, introduction of the gene may disrupt other favorable genetic characteristics. Replicated testing of families can begin in the F4 generation to improve the effectiveness of selection for traits with low heritability. At an advanced stage of inbreeding (i.e., F6 and F7), the best lines or mixtures of phenotypically similar lines are tested for potential release as new parental lines.

[0011] Backcross breeding has been used to transfer genes for a highly heritable trait into a desirable homozygous cultivar or inbred line which is the recurrent parent. The source of the trait to be transferred is called the donor parent. The resulting plant is expected to have the attributes of the recurrent parent (e.g., cultivar) and the desirable trait transferred from the donor parent. After the initial cross, individuals possessing the desired phenotype of the donor parent are selected and repeatedly crossed (backcrossed) to the recurrent parent. The process is used to recover all of the beneficial characteristics of the recurrent parent with the addition of the new trait provided by the donor parent.

[0012] Promising advanced breeding lines are thoroughly tested and compared to appropriate standards in environ-

ments representative of the commercial target area(s) for at least three or more years. The best lines are candidates for new commercial varieties or parents of hybrids; those still deficient in a few traits may be used as parents to produce new populations for further selection.

[0013] These processes, which lead to the final step of marketing and distribution, usually take from 8 to 12 years from the time the first cross is made and may rely on the development of improved breeding lines as precursors. Therefore, development of new cultivars is not only a time-consuming process, but requires precise forward planning, efficient use of resources, and a minimum of changes in direction. The results include novel genetic combinations not found in nature.

[0014] Some improvement of rice through breeding may be restricted to the natural genetic variation in rice and hybridizing species, such as wild rice. The introduction of new variation in a breeding program is usually through the crossing program as described, such as pedigree or backcross breeding. However, occasionally natural mutations are found that result in the introduction of new traits such as disease resistance or height changes. Breeders have also developed new traits by inducing mutations (small changes in the DNA sequence) into a rice genome. Some of these mutations or combination of genes are not found in nature. Commonly, EMS (Ethyl methanesulfonate) or sodium azide plus MNU (N-methyl-N-nitrosourea) are used as mutagenic agents. These chemicals randomly induce single base changes in DNA, usually of G and C changed to A and T. Overall effects are unpredictable. Most of these changes have no effect on the crop, because they fall either outside the gene coding regions or do not change the amino acid sequence of the gene product. However, some produce new traits or incorporate new DNA changes into previous lines.

[0015] The breeder has no direct control of mutation sites in the DNA sequence. The identification of useful changes is usually due to the random possibility that an effective mutation will be induced, and that the breeder will recognize the phenotypic effects of the change and will be able to select rice having that mutation for production. Seeds are treated with a mutagenic chemical and immediately planted to grow. The resulting plants are designated as M0 as the starting mutagenized population. M0, screened or not, can be selfed in autogamous crops like rice to produce M1 progeny, and subsequently M2, M3, and so forth, as the population is advanced. The M2 seed will carry numerous new variations; therefore, no two experiments will produce the same combinations. Among these variations new traits previously not existing in rice and previously unavailable for selection by a plant breeder may be found and used for rice improvement.

[0016] To find new traits the breeder must use efficient and targeted selection strategies because the process is completely random and has an extremely low frequency of useful new combinations. Among thousands of induced new genetic variants, there may be only one with a desirable new trait. An optimal selection system will screen through thousands of new variants and allow detection of a few or even a single plant that might carry a new trait. After identifying or finding a possible new trait, the breeder must develop a new cultivar by pedigree or backcross breeding and extensive testing to verify the new trait and cultivar exhibits stable and heritable value to rice producers. After a mutation is identified by whatever means, it may be transferred into rice by recombinant techniques.

Herbicide Resistance in Rice

[0017] Weeds in crop fields compete for resources and greatly reduce the yield and quality of the crop. Weeds have been controlled in crops through the application of selective herbicides that kill the weeds, but do not harm the crop. Usually selectivity of the herbicides is based on biochemical variations or differences between the crop and the weeds. Some herbicides are non-selective, meaning they kill all or almost all plants. Non-selective or broad spectrum herbicides can be used in crops only if the crops have a genetic mechanism that confers tolerance to the herbicides. Crops can be converted into "Herbicide Tolerance" (HT) if new genes are inserted that express specific proteins that convey tolerance or resistance to the herbicide. Resistance to herbicides has also been achieved in crops through genetic mutations that alter proteins and biochemical processes. These mutations may arise in nature, but in most crops identified they have been effected by the breeder.

[0018] In some instances, especially with repeated use of a particular herbicide, weeds have developed resistance through the unintended selection of natural mutations that provide resistance. When weeds become resistant to a particular herbicide, that herbicide is no longer useful for weed control. The development of resistance in weeds may be delayed through alternating the use of herbicides with different modes of action to control weeds, interrupting development of resistant weeds.

SUMMARY

[0019] Rice production is plagued by broad leaf and grass weeds that are difficult to control, amongst which there is a particularly hard to control weed called "red rice". One difficulty arises because red rice is so genetically similar to cultivated rice (they occasionally cross pollinate) that there are no selective herbicides available that target red rice, yet do not harm the cultivated rice. Control is currently provided in commercial rice production through the development of mutations found in rice that render rice resistant to broad spectrum herbicides e.g. imidazolinone and sulfonylurea herbicides. Rice crops resistant to herbicides that inhibit other deleterious plants, such as broad leaf plants, are needed.

[0020] Finding new mutations in rice that improve resistance to herbicides, would greatly benefit rice production. Obtaining and incorporating genes and combination of genes for improved herbicide resistance into rice genomes, while maintaining favorable characteristics to maintain or improve fitness, is challenging, unpredictable, time consuming and expensive, but necessary to meet the world's increasing food needs.

SUMMARY

[0021] Described and disclosed herein are novel and distinctive rice lines and hybrids with unique resistances to herbicides, in particular AHAS/ALS inhibiting herbicides.[1] The resistance conferred to rice plants by the substitutions A205V/G6254E in the AHAS/ALS amino acid sequence, when the rice plants are challenged by IMI herbicides, is significantly higher than that resistance/tolerance shown by

a rice plant carrying A205V alone or G654E alone. The uniqueness is due to a combination of genetic mutations leading to synergistic increases in herbicide tolerance/resistance in rice with the combination of genetic mutations, compared to levels of tolerance/resistance caused by the individual genetic mutations.

[1] For names of some suitable "IMI" herbicides, see "DEFINITIONS."

[0022] Various mutations in the nucleic acid coding sequence for the AHAS/ALS[2] enzyme cause amino acid substitutions that when expressed in rice result in an enzyme resistant to IMI tolerant herbicides. FIG. **1** shows alignment of nucleic acid sequences that encode amino acid sequences of AHAS/ALS in 2 rice lines, one (0001-2-2) with a mutation causing substitution A205V and another (80034-5) causing substitution G654E. Also shown are sequences in wild type rice (P1003) and R0146, a parental proprietary line. The designated amino acid substitutions encoded, are listed in boxes underneath the mutated codons. FIG. **2** shows positions of some of the AHAS/ALS known amino acid substitutions [205 (179), 653 (627), 654 (628)].[3]

[2] The gene coding for the acetohydroxyacid synthase protein (AHAS, EC2.2.1.6), also referred to as acetolactate synthase (ALS) in rice is present at a single locus in rice listed at locus LOC_Os06g51280 in the annotated database.

[3] Using both the Blackgrass and rice position numbers.

[0023] Stacking of both genetic mutations (designated RTC1 and RTC2)[4] is exemplified by the genomes of seeds from three hybrids deposited under the Budapest Treaty and designated in the ATCC as PTA-123859, PTA-123860, and PTA-123861 respectively (See Table 1).

[4] RTC1 caused the A205V amino acid substitution in the AHAS/ALS enzyme, wherein valine replaces alanine, and RTC2 caused the G654E substitution, wherein glutamic acid replaces glycine.

[0024] Unexpectedly, synergism exhibited in terms of IMI herbicide tolerance, is clearly greater than the sum of the dose/tolerance manifested by the AHAS protein carrying a single A205V or a single G654E mutation. Using data from multiple experiments, (identified as 155A-T11, 16-T7, 16GH-T7) conducted over 2 years, in multiple locations, with multiple different imidazolinone herbicides (imazetaphyr, imazamox and imazetapic) and multiple lines, it was estimated that the specific interaction of the two single mutations RTC1+RTC2, when acting together (RTC1−RTC2), and expressed as the additional avoidance of % Injury relative to the individual mutations, can be estimated as −25% reduction of rate of injury at doses of 1×, −55% reduction in injury at 2×, −65% at 3× and −67% reduction at 4× (FIG. **10**A).

[0025] It is a surprising and unexpected result to find herbicide resistance/tolerance affected by a combination of mutations leading to two different substitutions in a target enzyme, as disclosed herein, AHAS/ALS. It is particularly unexpected that the combination produces a synergistic response—less percent injury due to herbicide exposure in rice with the combination, than in rice with either mutation alone. Importantly, yield was not deleteriously affected, as has been reported attending some mutant herbicide resistant/tolerant rice.

[0026] Because these mutations affect the same enzyme and are in the same gene, one might have expected at most an additive effect, and likely a negative effect on the enzyme. Unexpectedly, synergism in the level of herbicide tolerance resulted, without deleterious effects on other traits (Tables 3, 4).

[0027] The mechanism of herbicide tolerance has been classified roughly into two groups: target-site and non-target-site herbicide tolerance. Target-site herbicide tolerance is caused by the prevention of herbicide binding to the target enzyme, caused by point mutations occurring in the target. The molecular mechanisms of target-site herbicide tolerance are regulated mostly by a single gene encoding a target enzyme harboring a point mutation. The hybrids disclosed herein, have a combination of 2 point mutations.

[0028] Disclosed herein is a method to control weeds in a rice field, wherein the rice in the field includes plants resistant to IMI herbicides. The method includes:

[0029] a. using herbicide resistant/tolerant rice in the field; and

[0030] b. contacting the rice field with at least one herbicide, or a plurality of herbicides, for example, any that may belong to the family of chemicals known as AHAS/ALS-inhibitors, or any that may belong to the class of herbicides known as AHAS/ALS-inhibiting herbicides.

[0031] A method for growing herbicide resistant/tolerant rice plants includes (a) planting resistant rice seeds; (b) allowing the rice seeds to sprout; (c) applying one or more herbicides to the rice sprouts at levels of herbicide that would normally inhibit the growth of a rice plant.

[0032] Methods of producing herbicide-tolerant rice plants may also use a transgene or plurality of transgenes. One embodiment of such a method is transforming a cell of a rice plant with transgenes, wherein the transgenes encode 2 different mutations each leading to rice resistant to IMI inhibitors. Any suitable cell may be used in the practice of these methods, for example, the cell may be in the form of a callus. Specific mutations disclosed herein include those leading to substitutions A205V and G654E in the AHAS/ALS enzyme. With this combination, synergism in resistance was effected.

BRIEF DESCRIPTION OF DRAWINGS

[0033] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the office upon request and payment of the necessary fee.

[0034] FIG. **1** shows an alignment of nucleic acid sequences among wild type rice line P1003 (SEQ ID NO: 1), proprietary parental line R0146 (SEQ ID NO: 3), and one line that has a mutation encoding a substitution A205 (179)V (SEQ ID NO: 2) and another line with G654 (628)E (SEQ ID NO: 4).

[0035] FIG. **2** shows the AHAS/ALS amino acid sequence alignment of substitutions at positions 205 (SEQ ID NO: 5), 653 (SEQ ID NO: 6) and 654 (SEQ ID NO: 7); where P1003 (SEQ ID NO: 10) and R0146 (SEQ ID NO: 11) are unmutated rice lines; also shown are Nipponbare (SEQ ID NO: 12) and Arabidopsis (SEQ ID NO: 13). FIG. **2** also discloses SEQ ID NOS: 8-9, respectively, in order of appearance (Cyp-MT-653, Cyp-WT). Blanks show non-alignment.

[0036] FIG. **3**A to FIG. **3**I illustrate the results of Example 1: FIG. **3**A control; FIG. **3**B 0.5× Imazamox; FIG. **3**C 1× Imazamox; FIG. **3**D 2× Imazamox; FIG. **3**E 4× Imazamox; FIG. **3**F 1× Imazethapyr +0.5× Imazamox; FIG. **3**G 1× Imazethapyr+1× Imazamox; FIG. **3**H 1× Imazethapyr+2× Imazamox; FIG. **31** 1× Imazethapyr+4× Imazamox; Pots L to R: LF2-RTC2/LM1-RTC1, LF1-RTC2/LM1-RTC1, LF3-RTC2\LM4-RTC1, mutated line P1003A205V, mutated line

R0146G654E, sensitive control commercial variety Cypress, commercial hybrid CLXL745.

[0037] FIG. **4** graphically illustrates the results of Example 1.

[0038] FIG. **5**A to FIG. **5**D illustrate the results of Example 2: FIG. **5**A; Control; FIG. **5**B 8×; FIG. SC 1×; FIG. SD; 0.25×; Planting order L to R: P1003A205V, R0146G654E, P1003, LF2-RTC2/LM1-RTC1, LF3-RTC2\LM4-RTC 1.

[0039] FIG. **6** graphically illustrates the results of Example 2.

[0040] FIG. **7** graphically illustrates the results of Example 3.

[0041] FIG. **8** is a graphical illustration of percent injury 4 weeks post preflood application.

[0042] FIG. **9** is a graphical comparison of yield after various doses of IMI herbicides: control compared to various rice lines and hybrids.

[0043] FIG. **10**A and FIG. **10**B show average percent injury 3 weeks post last herbicide application across trials [15SA-T11, 16-T7, 16GH-T7], which included independent experiments with single active ingredients imazetaphyr, imazamox or imazetapic, and combinations in sequential applications; Data on effects was averaged across experiments for each line, RTC1 only, RTC2 only or RTC1−RTC2; Synergistic effects of RTC1+RTC2 interactions are expressed as observed RTC1+RTC2%Injury minus expected RTC1+RTC2% Injury calculated as [(RTC1+RTC2)-(RTC1XRTC2/100)]; FIG. **10**A shows average % injury 3 weeks post herbicide application and RTC1−RTC2 synergistic effects calculated as stated above; FIG. **10**B graphically shows percent average % injury, at different application doses, for the homozygous single mutation lines, RTC1 and RTC2, and the double hemizygous RTC1/RTC2.

[0044] FIG. **11** shows developmental stages of rice.

DETAILED DESCRIPTION

[0045] Rice lines having different herbicide resistance genes, either pyramided or

[0046] stacked in the same genetic background or, as single products that are used alternatively in the rotation used by the farmer, represent a critical tool or strategy in extending the useful life of herbicides because these practices slow the development of herbicide resistant variants among the targeted weeds. Several methods are possible to deploy these resistances into hybrids or varieties for weed control, as well as options for hybrid seed production. The rice lines described herein represent new methods for weed control in rice and can be deployed in any of many possible strategies to control weeds and provide for long-term use of these and other weed control methods.

The Novel Effect of Dual Mutations on The AHAS Protein

[0047] AHAS is an important enzyme in plants and microorganisms that catalyzes the formation of acetolactate from pyruvate, the first step in the biosynthesis of aminoacids valine and isoleucine. The functional protein complex can have a homodimer or homotetramer structure, and presents both large catalytic subunits and small regulatory subunits.

[0048] The regulatory subunit stimulates activity of the catalytic subunit and confers sensitivity to feedback inhibition by branched-chain amino acids. Because the AHAS crystalline structure is well characterized for Arabidopsis thaliana, it has been possible to (1) identify the AHAS-herbicide binding sites (2) establish and understand the molecular interaction between AHAS, its cofactors and the herbicides that affect it. The AHAS catalytic site is deep within a channel of the protein, but it is noteworthy that AHAS herbicides do not bind within the catalytic site. Rather, they bind across an herbicide binding domain that straddles the channel entry, thereby blocking substrate access to the catalytic site and interrupting normal enzyme metabolism causing death to the plant. Across this domain, many amino acid residues are involved in herbicide binding. Structurally different AHAS herbicides orientate differently in the herbicide binding domain, causing a variable level of interaction for any given substitution. Specific amino acid substitutions within the herbicide binding domain can confer resistance to some, but not to other, AHAS herbicides (see review in Powles and Yu, 2010).

[0049] Despite the large number of well characterized single induced or spontaneous mutations across many of the amino acid residues, none have been reported describing simultaneous substitutions at 2 or more active substitutions at critical amino acid residues of the AHAS gene. Also, given the proximity of these different active herbicide binding sites, it is also impossible to stack these from single mutation donors into a single background genome, by means of sexual reproduction. This limitation exists simply on account of the extraordinary improbability of such a specific recombination event. Thus, the novelty of the RTC1−RTC2 product which effectively overcomes the otherwise improbable combination of multiple mutations in the AHAS protein, in hybrid rice disclosed herein. The hybrids were produced by combining the two different mutated alleles at the AHAS locus, which in turn, produces a functional protein complex that contains subcomponents of both forms. Surprisingly the result is to confer on the rice, synergistic herbicide tolerance when compared with the pure form single mutation effects.

Applications of Rice with 2 Different Mutations

[0050] Cells derived from herbicide resistant seeds, plants grown from such seeds and cells derived from such plants, progeny of plants grown from such seed and cells derived from such progeny are within the scope of this disclosure. The growth of plants produced from deposited seeds, and progeny of such plants will typically be resistant/tolerant to herbicides, e.g. an IMI inhibitor at levels of herbicides that would normally inhibit the growth of a corresponding wild-type plant. There are some natural (non-induced) levels of tolerance to some herbicides, but they are not capable of protecting plants at levels that would be commercially useful.

[0051] A method for controlling growth of weeds in the vicinity of herbicide resistant/tolerant rice plants is also within the scope of the disclosure. One example of such methods is applying one or more herbicides to the fields of rice plants at levels of herbicide that would normally inhibit the growth of a rice plant. For example, at least one herbicide inhibits AHAS/ALS activity.

[0052] In order to maximize weed control in a rice field, different herbicides may be required to cover the spectrum of weeds present and, in turn, several applications along the crop cycle may be required for any one particular herbicide depending on the overlap between the window of effective

control provided by a single application and the window of time during which its target weed may germinate, which often is longer than the protection afforded by a single herbicide application. Temperature, and soil moisture conditions are key factors that affect both window of herbicide efficacy, window of moment of weed germination and growth. Based on these factors, herbicide control models often include sequential repeated application during the crop cycle.

[0053] In a standard herbicide tolerance system, for example, one currently used commercially in rice, for resistance to imidazolinone herbicides, the first application of the herbicide is applied at the 2 leaf stage, with the second application following a minimum of 10 days later just prior to the establishment of permanent flood when the plants are tillering. The purpose of the second application is to eliminate weeds that may have germinated after the first application before they can be effectively suppressed by flooding. In some traits, including "IMI" inhibitor herbicides, the timing of herbicide applications can be critical not only for effective weed control, but also for the level of tolerance observed in the plants themselves. Plant injury observed in response to herbicide application may align closely with plant stage. In some rise lines, very early post-emergence applications cause much higher injury at the 1 leaf stage, with observed injury declining at each growth stage of the plant through first tiller. Some herbicide tolerance traits even exhibit no tolerance to pre-emergent applications even though post-emergence tolerance is excellent. This variable herbicide response linked to plant growth stage requires careful testing to establish the boundaries of safe usage of a new herbicide tolerant product.

[0054] A plurality includes, for example, at least 2 "IMI" herbicides.

[0055] In considering combinations of different herbicide resistance genes, irrespective of whether the combination includes two or more different modes of action for the same herbicide, or two or more genes for herbicides of different families or functions, antagonistic or synergistic interactions may be observed resulting from gene to gene interactions, as some of the embodiments described herein have evidenced. The combination disclosed herein of the novel mutated genes resistant to AHAS/ALS-inhibiting herbicides results in herbicide tolerance that is far superior to the additive resistance of the two genes acting individually, demonstrating synergism. (FIGS. **3-6**; Examples 1-4).

[0056] Rice production for good yields requires specific weed control practices. Some herbicides are applied as premergents, after planting but before crop emergence; other as postemergents. In the case of rice, postemergent application can be before the crops are flooded, of after. Preferred applications are normally times, according to the developmental stage of the crop, as defined by the number of open leaves in the growing plant (FIG. **11**: Developmental Stages of Rice). Timing of herbicide applications is an important factor, not only from the perspective of maximizing the efficiency of weed control, but also from the perspective of minimizing impact on the herbicide tolerant crop. This consideration stems from the fact that mutagenized, naturally occurring or transgenic herbicide resistances often are not completely independent of dose and application timing effects. Different genes of herbicide resistance have different dose responses, as well as timing of application responses whereby, typically, phytotoxity in the resistant crop increases as dose increases beyond a certain level, or phytotoxicity to the resistant crop varies with varying timings of application for a given herbicide dose.

[0057] Evaluation of the novel herbicide resistance genes, subject of this application, was conducted with a range of suitable herbicide doses, that cover application rates typically used for rice farming operations while also taking into consideration possible deviations from the manufacturer-recommended doses. Considering 1×, the recommended manufacturers or best practice recommended dose, the most frequently evaluated additional doses are 2× and 4× with some experiments including other values.

[0058] The RTC1 line was derived from rice line P1003. RTC2 was derived from R0146—a proprietary line of Chinese origin. Neither of these two lines has any tolerance to imidazolinone herbicides. After backcrossing to fix the mutations and remove unwanted effects, the traits have been introgressed into a number of inbred rice lines in order to produce a range of hybrid rice varieties suited to a range of different commercial requirements for herbicide tolerance.

[0059] The A179(205)V mutation was developed by EMS mutagenesis from the line P1003, also called Lemont, which is the public variety designated Cypress. The independently developed mutation G628(654)E was obtained by chemical mutagenesis process (Sodium Azide+MNU) from the proprietary line R0146 of Chinese origin. These mutations were independently fixed by inbreeding during the line optimization process following the mutagenesis and early detection, and are therefore available in stable homozygous configuration in the derived inbred lines. These two independent mutations, being localized in the same expressed gene at positions contained within the same protein, are not stacked in an inbred stock. Fortunately, hybrid products with one dose of each allele and expressing both modifications show higher herbicide tolerance than homozygous lines for either gene and are, therefore, the targeted product.

[0060] All genetic materials used in the development of these mutants, or derived therein, are the property of RiceTec. All markers used were internally developed from available public sequences or from sequence information derived from the same materials. Standard IMI commercial herbicides were selected for the screening process, using label guidance to determine herbicide use parameters. Herbicide response was determined using plant injury rates (See TABLE 2).

[0061] TABLE 3 is a comparison of morphological-physiological/grain quality attributes of the RTC1−RTC2 hybrid rice lines, compared to their non-mutated counterparts, to highlight that these mutant IMI tolerant rice lines are otherwise agronomically identical to their non-mutated counterparts. Overall, there were no commercially relevant differences identified between the RTC1−RTC2 rice hybrids and their un-mutated controls (same original line lacking the mutations). Comparison of hybrids containing both mutations with the single mutation and with the control line also showed few statistically significant differences, none of which are biologically relevant. It is known that mutagenic treatments often result in multiple changes in the regenerated plants, but these lines have been repeatedly backcrossed and converted into inbred lines thereby removing unwanted mutations from the germplasm.

[0062] The examples below are illustrative of the invention, but not limiting.

EXAMPLES

Example 1

16GH-T7: Imazamox Trial (see FIG. 3A to FIG. 3I and FIG. 4)

[0063] FIG. **3** Trial setup: Trial consisted of 2 treatment regimens with Imazamox

[0064] applications at approximately the preflood stage with rates of 0.5×, 1×, 2×, or 4×, either alone or 2 weeks following application of 1× Imazethapyr.

[0065] FIG. **4** Representative pots 4 weeks post application of Imazamox.

[0066] Summary: The results of this trial indicates that RiceTec (RT) IMI hybrids show high levels of tolerance to Imazamox herbicide. No injury was observed in any of the RT IMI hybrids in any treatment. However, injury was observed in the single trait mutant lines. This was expected, because it is known that the G654E mutation only confers a weak level of tolerance to IMI herbicides. Due to the trial being conducted in the greenhouse during the difficult winter season the injury response may not have shown exactly as that of an optimal field trial, but given the consistency of response across all treatments the data indicates that RT IMI hybrids have equal or higher levels of tolerance to Imazamox than current commercial IMI hybrids.

Example 2

16-T7: Log Sprayer Trial; Imazethapyr (see FIG. 5A, 5B, 5C, 5D; and FIG. 6)

[0067] Trial: single herbicide application at the 3-4 leaf stage using the half-step log sprayer at rates from 16× to $\frac{1}{128}$×.

[0068] Plot images 3 weeks post application. FIG. **5**A Control, FIG. **5**B 8×, FIG. **5**C 1×, FIG. **5**D 0.25×. Planting order L to R: P1003A205V, R0146G654E, P1003, P1003, LF2-RTC2/LM1-RTC1, LF3-RTC2\LM4-RTC1.

[0069] Summary: FIG. **6** shows % of injury 3 weeks post 3-4 leaf application. The homozygous A205V (RTC1) mutant line (P1003A205V) had very good tolerance to even high rates of Imazethapyr herbicide. Observed injury in the P1003A205V was below 10% at all tested rates 8× (48 oz/acre) and lower. Recorded injury in the G654E (RTC2) homozygous line was lower than expected, but this may be partially due to the later application. The G654E/A205V hybrids showed very strong levels of tolerance in this trial, with no observed injury by three weeks post application in either hybrid.

Example 3

15SA-T9: IMI Conversion Efficacy Trial of Recent IMI Conversions (see FIG. 7)

[0070] Trial: 2 leaf and preflood application of IMI herbicide on materials converted with RiceTec IMI traits and controls. All males included RTC1 (A205V) in their genome. Females with RTC2 (G654E) were not included due to limited seed numbers. Females were tested separately in 15GH-T8 using potted plant replications.

[0071] Summary: A205V-G654E hybrids had very low injury rates in response to 2 applications of Imazethapyr. The injury observed in the RT IMI A205V-G654E hybrids was comparable to current commercial IMI hybrids. This trial supports the commercialization of the A205V-G654E hybrid. This trial was maintained through harvest.

Example 4

155A-T11: KIFIX Trial (see FIG. 8 and FIG. 9)

[0072] Trial: 2 leaf and preflood application of Imazethapyr or Kifix™ (imazapic/imazapir) herbicide on materials converted with RiceTec IMI traits and commercial IMI control.

[0073] Summary: Injury rates observed in the converted A205V-G654E hybrids is very similar between Imazethapyr and Kifix (imazapic/imazapir) applications with slightly reduced injury observed in the equivalent Kifix treatments. Consistent with previous data, the heterozygous A205V hybrids have reduced tolerance to both herbicides as compared to the A205V-G654E hybrids. Yields of the converted hybrids was surprising in that all three of the two trait converted hybrids (A205V-G654E) performed even better than the commercial Clearfield® control (CLXL745) when herbicide was applied, with the internally developed two trait hybrids actually showing more resistance to yield loss at 4× application rates than the Clearfield® hybrid control. This supports that IMI traits prove effective in conferring tolerance to both Newpath™ for the US market as well as Kifix for Brazil with no significant yield penalty.

[0074] Overall, FIGS. **10**A and **10**B show average percent injury 3 weeks post last herbicide application across trials [155A-T11, 16-T7, 16GH-T7], which included independent experiments with single active ingredients imazetaphyr, imazamox imazetapic, in sequential applications, or unique active ingredients or in combination. Also averaged across material carrying RTC1 only, RTC2 only or RTC1−RTC2. Synergistic effects of RTC1+RTC2 interactions expressed as observed RTC1+RTC2% Injury minus expected RTC1+RTC2% Injury calculated as [(RTC1+RTC2)-(RTC1XRTC2/100)]. (See Table 2 for Injury Rating Scale.)

Seed Deposits Under Budapest Treaty

[0075] Seed deposits of resistant/tolerant rice hybrids were deposited by RiceTec Inc. in the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va 20110, United States of America on Feb. 1, 2017. PTA accession numbers are PTA-123859, PTA-123860 and PTA-123861. (See also TABLE 1.) All restrictions will be removed upon granting of a patent, and the deposits are intended to meet all of the requirements of 37 C.F.R. § § 1.801-1.809, and satisfy the Budapest Treaty requirements. The deposit will be maintained in the depository for a period of thirty years, or five years after the last request, or for the enforceable life of the patent, whichever is longer, and will be replaced as necessary during that period.

Definitions

[0076] In the description and tables which follow, a number of terms are used. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided:

[0077] Allele. Allele is any one of many alternative forms of a gene, all of which generally relate to one trait or

characteristic. In a diploid cell or organism, the two alleles of a given gene occupy corresponding loci on a pair of homologous chromosomes.

[0078] Backcrossing. Process of crossing a hybrid progeny to one of the parents, for example, a first generation hybrid F1 with one of the parental genotypes of the F1 hybrid.

[0079] Blend. Physically mixing rice seeds of a rice hybrid with seeds of one, two, three, four or more of another rice hybrid, rice variety or rice inbred to produce a crop containing the characteristics of all of the rice seeds and plants in this blend.

[0080] Cell. Cell as used herein includes a plant cell, whether isolated, in tissue culture or incorporated in a plant or plant part.

[0081] Cultivar. Variety or strain persisting under cultivation.

[0082] Derived. As used herein means that a gene or plurality of genes is taken, obtained, received, traced, replaced or descended from a source plant or seeds, and regardless of the method used, was transferred to a different plant.

[0083] Embryo. The embryo is the small plant contained within a mature seed.

[0084] Essentially all the physiological and morphological characteristics. A plant having essentially all the physiological and morphological characteristics of the hybrid or cultivar, except for the characteristics derived from the introduced gene of interest.

[0085] Grain Yield. Weight of grain harvested from a given area. Grain yield could also be determined indirectly by multiplying the number of panicles per area, by the number of grains per panicle, and by grain weight.

[0086] Imidazolinone. "IMI" includes for example imazapyr, imazapic, imazethapyr, imazamox, imazamethabenz and imazaquin.

[0087] Injury to Plant. Is defined by comparing a test plant to controls and finding the test plant is not same height; an abnormal color, e.g. yellow not green; unusual leaf shape, curled, fewer tillers; does not survive (see Table 2).

[0088] Induced. As used herein, the term induced means genetic resistance appeared after treatment with a mutagen.

[0089] Introgress. As used herein, moving genes from a plant to another, so that the plant and its progeny carry the gene.

[0090] Locus. A locus is a position on a chromosome occupied by a DNA sequence; it confers one or more traits such as, for example, male sterility, herbicide tolerance, insect resistance, disease resistance, waxy starch, modified fatty acid metabolism, modified phytic acid metabolism, modified carbohydrate metabolism and modified protein metabolism. The trait may be, for example, conferred by a naturally occurring gene introduced into the genome of the variety by backcrossing, a natural or induced mutation, or a transgene introduced through genetic transformation techniques. A locus may comprise one or more alleles integrated at a single chromosomal location.

[0091] Non-induced. As used herein, the term non-induced means genetic resistance not known to be induced; may be at different location in the genome, than an induced resistance.

[0092] Plant. As used herein, the term "plant" includes reference to an immature or mature whole plant, including a plant from which seed or grain or anthers have been removed. Seed or embryo that will produce the plant is also considered to be the plant.

[0093] Plant Part. As used herein, the term "plant part" (or a rice plant, or a part thereof) includes protoplasts, leaves, stems, roots, root tips, anthers, seed, grain, embryo, pollen, ovules, cotyledon, hypocotyl, glumes, panicles, flower, shoot, tissue, cells, meristematic cells and the like.

[0094] Progeny. Descendants of source plants obtained by breeding, recombinant or other methods, wherein genes of interest are replicated from the source plants in the descendent genes.

[0095] Pyramided. Vector stacks: when different traits are stacked in a vector, and in a single act or transformation, the traits are transmitted to the plant; the transformed plant exhibits multiple traits. Pyramiding traits: sequential transformation of single traits (all vectors are single trait carriers), or alternatively each trait is transformed in parallel, onto the same line and simple sexual crossing is used to "pyramid" them into a single line. "Either added gradually (from different donors) or added all at once (from a single multi-trait donor)."

[0096] Quantitative Trait Loci (QTL). Genetic loci that controls to some degree numerically measurable traits that are usually continuously distributed.

[0097] Recombinant/Non-Recombinant. If non-parental combination occurs, a rice patent is recombinant.

[0098] Regeneration. Regeneration refers to the development of a plant from tissue culture.

[0099] Resistance/Resistant.[5] The inherited ability of a plant to survive and reproduce following exposure to a dose of herbicide normally lethal to the wild type resistance may be naturally occurring or induced by such techniques as genetic engineering or selection of variants produced by tissue culture or mutagenesis.

[5] Weed Science Society of America, Weed Technology, vol. 12, issue 4 (October-December, 1998, p. 789)

[0100] Single Gene Converted (Conversion). Single gene converted (conversion) includes plants developed by a plant breeding technique called backcrossing wherein essentially all of the desired morphological and physiological characteristics of an inbred are recovered, while retaining a single gene transferred into the inbred via crossing and backcrossing. The term can also refer to the introduction of a single gene through genetic engineering techniques known in the art.

[0101] Source Plant or Seed. A plant or seed from which a gene or plurality of genes, is transferred to a different plant, seed, callous or other suitable recipient.

[0102] Stacking. Adding more than one thing to the same receiving entity. Methods of achieving the "stacked" state include: methods of vector-stack two or more genes in a single vector and do a single transformation to achieve stack; do sequential transformations into same receptor adding traits stepwise; achieve stacked hybrid simply by end crossing parentals carrying different traits; develop lines with multiple traits by sequential mutagenesis or crossing, and fixing the stacked state into one parent; and variants thereof.

[0103] Synergism. As described in the *Herbicide Handbook* of the Weed Science Society of America, Ninth Edition, 2007, p. 429, "'synergism' [is] an interaction of two or

more factors such that the effect when combined is greater than the predicted effect based on the response to each factor applied separately."

[0104] The following equation may be used to calculate the expected resistance/tolerance in rice with combinations of mutations to herbicides, e.g., A and B:

$$\text{Expected}=A+B-(A\times B/100)$$

[0105] A=observed efficacy of mutation A at the same concentration of herbicide;

[0106] observed efficacy of mutation B at the same concentration of herbicide.

[0107] Synergistic in the herbicide context can mean that the use of herbicide results in an increased weed control effect compared to the weed control effects of A+B that are possible with the use of each herbicide alone. Or synergistic may be considered as the resistance/tolerance level of the rice, with combined mutations (stacked) compared to effects of a rice with a single mutation.

[0108] In some embodiments, the damage or injury to the undesired vegetation caused by the herbicide is evaluated using a scale from 0% to 100%, when compared with the untreated control vegetation, wherein 0% indicates no damage to the undesired vegetation and 100% indicates complete destruction of the undesired vegetation.

[0109] Tolerance/Tolerant. The inherent ability of a species to survive and

[0110] reproduce after herbicide treatment implies that there was no selection or generic manipulation to make the plant tolerant.

[0111] Resistance/tolerance are used somewhat interchangeably herein; for a specific rice plant genotype information is provided on the herbicide applied, the strength of the herbicide, and the response of the plant.

Publications Cited

[0112] Powles, Stephen B. and Yu, Qin, "Evolution in Action: Plants Resistant to Herbicides," Annu. Rev. Plant Biol. (2010) 61:317-347.

TABLE 1

Hybrid Rice with Both RTC1 and RTC2 in Their Genomes

Source #	Hybrid	Designation	PTA
15USAA04803	S5120G654E/P1062A205V	TH1524551	123860
15USAA04858	S5209G654E/P1308A205V	TH1524568	123859
15USAA04810	S5107G654E/P1062A205V	TH1524558	123861

TABLE 2

Herbicide Injury Rating Scale in Rice

Score	Rating description
0	no visible injury
1	injury observed in at least 1 plants but very minimal
5	minimal injury observed across plot
10	plants are stunted 10% as compared to control, or plants show herbicide injury on approximately 10% of leaf area in the plot
15	plants are stunted 15% as compared to control, or plants show herbicide injury on approximately 15% of leaf area in the plot
20	plants are stunted 20% as compared to control, or plants show herbicide injury on approximately 20% of leaf area in the plot
25	plants are stunted 25% as compared to control, or plants show herbicide injury on approximately 25% of leaf area in the plot
30	plants are stunted 30% as compared to control, or plants show herbicide injury on approximately 30% of leaf area in the plot
35	plants are stunted 35% as compared to control, or plants show herbicide injury on approximately 35% of leaf area in the plot
40	plants are stunted 40% as compared to control, or plants show herbicide injury on approximately 40% of leaf area in the plot
45	plants are stunted 45% as compared to control, or plants show herbicide injury on approximately 45% of leaf area in the plot
50	plants are stunted 50% as compared to control, or plants show herbicide injury on approximately 50% of leaf area in the plot
55	plants show herbicide injury on approximately 55% of leaf area in the plot
60	plants show herbicide injury on approximately 60% of leaf area in the plot
65	plants show herbicide injury on approximately 65% of leaf area in the plot
70	plants show herbicide injury on approximately 70% of leaf area in the plot
75	plants show herbicide injury on approximately 75% of leaf area in the plot
80	plants show herbicide injury on approximately 80% of leaf area in the plot
85	plants show herbicide injury on approximately 85% of leaf area in the plot
90	plants show herbicide injury on approximately 90% of leaf area in the plot
95	All plants severely injured, most are dead. Some green tissue spread throughout plot.
99	nearly all plants are dead, but at least 1 plant has green tissue.
100	all plants dead and brown. No green tissue in the plot.

TABLE 3

Yield, Grain Quality and Maturity information for deposited ATCC RTC1-RTC2 hybrids and controls

	Hybrid/Controls					
	LF2-RTC2/ LM1-RTC1	LF2/ LM1-CL	LF1-RTC2/ LM1-RTC1	LF1/ LM1	LF3-RTC2\ LM4-RTC1	LF3\LM4
ATCC#	PTA-123860		PTA-123861		PTA-123859	
Yield (lbs/ac)	10128.38	10234.75	9189.8	9930.19	10187.72	9967.7
Lodging %	17.4	20.3	22.8	19.7	13	10.0
Days to 50% Heading	82.75	81.63	81.7	83.6	86.33	80.3
Plant Height (inches)	114.56	115.44	122.9	114.46	123.7	122.9
Total Mill %	71.9	72.6	71.3	72	71.3	70.0
Whole Mill %	60.5	60.4	62.9	62.7	64.7	60.0
Grain Length (mm)	6.72	6.84	6.88	6.66	5.48	5.6
Grain Width (mm)	2.08	2.1	2.12	2.1	2.6	2.7
Length Width Ratio	3.23	3.26	3.24	3.17	2.11	2.1
FIGS. %	6.25	3.2	2.9	6.25	2.43	1.9
White Belly %	13.43	13.35	10.35	12.62	5.23	4.5
Amylose	19.8	19.9	20.3	19.8	15.4	15.5
ASV	3.4	3.1	4.7	3.2	5.0	4.3
Moisture %	15.7	16.6	17.8	16.8	17.3	17.9
Grain Type	Long	Long	Long	Long	Medium	Medium

Material includes: ATTC submissions PTA-123860 [LF2-RTC2/LM1-RTC1], PTA-123861 [LF 1-RTC2/LM1-RTC1], PTA-123859 [LF3-RTC2\LM4-RTC1], and their respective controls LF2/LM1-CL, LF1/LM1 and LF3\LM4. Multi-location yield performance and grain quality data is provided to demonstrate equivalency of RTC1–RTC2 products to controls carrying the same genetic base. All materials were sprayed at 2 leaf stage with 1× application of imazamox.

TABLE 4

Multi-location yield and grain quality evaluation for additional RTC1-RTC2 material compared with currently commercial ClearField ® version of same hybrids. All materials were sprayed at 2 leaf stage with 1X application of imazamox.

Material	Year	Locations	Yield cyWa	Days To Heading	Height (cm)	Grain Retention	Whole milling %	Chalk %	White Belly Chalk %	Amylose	Gel Temp.
LF2/LM1-CL	2017	8	10235	83	115	Good	58	4	15	19	Interm.
LF2-RTC2/ LM1-RTC1	2017	8	10128	84	115	Good	59	7	15	19	Interm.
LF2/LM1-CL	2016	5	9295	80	121	Good	59	5	12	19	Interm.
LF2-RTC2/ LM1-RTC1	2016	5	8976	81	128	Good	57	7	15	19	Interm.
HYB5-CL	2017	5	9401	83	115	Good	59	7	17		Interm.
HYB5MG-FP	2017	5	9189	81	123	Excellent	58	5	14		Interm.
HYB5-CL	2016	5	8897	85	122	Good	58	8	13	19	Interm.
HYB5MG-FP	2016	5	8726	84	124	Good	56	8	15	20	Interm.
HYB5-CL	2016	7	8897	85	122	Poor	58	8	13	19	Interm.
HYB5-FP	2016	7	8726	84	124	Poor	56	8	15	20	Interm.
HYB4-CL	2017	7	10236	88	119	Good	60	7	14		Interm.
HYB4	2017	7	10666	85	121	Good	59	8	16		Interm.
HYB4-FP	2017	7	10739	85	119	Excellent	59	8	15		Interm.

Material includes: control commercial hybrids with ClearField® herbicide tolerance (CL suffix) and comparative pre-commercial hybrids with same parental source but carrying the RTC1–RTC2 (FP suffix) herbicide tolerance mutations. Clearfield® hyrbrids carry the IMI mutation localized in position 653.

SEQUENCE LISTING

```
Sequence total quantity: 13
SEQ ID NO: 1            moltype = DNA  length = 2878
FEATURE                 Location/Qualifiers
source                  1..2878
                        mol_type = genomic DNA
```

-continued

organism = Oryza sativa

SEQUENCE: 1

```
tgtaccctcc acccgttatt gggasggatc cacctgtcat cctcatccta tcaactgaca  60
cgcgggccca gatcgacccc gacgtggcag tgtgtcatcc tatcccaccg acatctgggg  120
cccaccgtga cgtggcccac acgatcccat ccgagccaca catcgcctca cgctgcgtca  180
ccgcgcgcgg acaaaacacc cacaccccca cactctccac ccctctctct ctctctcgcc  240
caaacccaga aaccctcgcc gccgccgccg ccgccgccgc catcacccac catggctacg  300
accgccgcgg ccgcggccgc caccttgtcc gccgccgcga cggccaagac cggccgtaag  360
aaccaccagc gacaccacgt ccttcccgct cgaggccggg tgggggcggc ggcggtcagg  420
tgctcggcgg tgtccccggt caccccgccg tccccggcgc cgccggccac gccgctccgg  480
ccgtgggggc cggccgagcc ccgcaagggc gcggacatcc tcgtggaggc gctggagcgg  540
tgcggcgtca gcgacgtgtt cgcctacccg ggcggcgcgt ccatggagat ccaccaggcg  600
ctgacgcgct ccccggtcat caccaaccac ctcttccgcc acgagcaggg cgaggcgttc  660
gcggcgtccg ggtacgcgcg cgcgtccggc cgcgtcgggg tctgcgtcgc cacctccggc  720
cccggggcaa ccaacctcgt gtccgcgctc gccgacgcgc tgctcgactc cgtcccgatg  780
gtcgccatca cgggccaggt cccccgccgc atgatcggca ccgacgcctt ccaggagacg  840
cccatagtcg aggtcacccg ctccatcacc aagcacaatt accttgtcct tgatgtggag  900
gacatccccc gcgtcataca ggaagccttc ttcctcgcgt cctcgggccg tcctggcccg  960
gtgctggtcg acatccccaa ggacatccag cagcagatgg ctgtgccagt ctgggacacc  1020
tcgatgaatc taccggggta cattgcacgc ctgcccaagc cacccgcgac agaattgctt  1080
gagcaggtct tgcgtctggt tggcgagtca cggcgcccga ttctctatgt cggtggtggc  1140
tgctctgcat ctggtgatga attgcgccgg tttgttgagc tgaccggcat cccagttaca  1200
accactctga tgggcctcgg caatttcccc agtgatgatc cgttgtccct gcgcatgctt  1260
gggatgcatg gcacggtgta cgcaaattat gcggtggata aggctgacct gttgcttgca  1320
tttggcgtgc ggtttgatga tcgtgtgaca gggaaaattg aggcttttgc aagcagggcc  1380
aagattgtgc acattgacat tgatccagcg gagattggaa agaacaagca accacatgtg  1440
tcaatttgcg cagatgttaa gcttgcttta cagggcttga atgctctgct agaccagagc  1500
acaacaaaga caagttctga ttttagtgca tggcacaatg agttggacca gcagaagagg  1560
gagtttcctc tggggtacaa gacttttggt gaagagatcc caccgcaata tgctattcag  1620
gtgctggatg agctgacgaa aggggaggca atcatcgcta ctggtgttgg acagcaccag  1680
atgtgggcgg cacaatatta cacctacaag cggccacggc agtggctgtc ttcggctggt  1740
ctgggcgcaa tgggatttgg gctgcctgct gcagctggtg cttctgtggc taacccaggt  1800
gtcacagttg ttgatattga tggggatggt agcttcctca tgaacattca ggagttggca  1860
ttgatccgca ttgagaacct cccggtgaag gtgatggtgt tgaacaacca acatttgggt  1920
atggttgtgc aatgggagga taggttttac aaggcaaata gggcgcatac atacttgggc  1980
aacccagaat gtgagagcga gatatatcca gattttgtga ctattgctaa agggttcaat  2040
attcctgcag tccgtgtaac aaagaagagt gaagtccgtg ccgccatcaa gaagatgctc  2100
gagaccccag ggccatactt gttggatatc atcgtcccac accaggagca tgtgctgcct  2160
atgatcccaa gtgggggcgc attcaaggac atgatcctgg atggtgatgg caggactatg  2220
tattaatcta taatctgtat gttggcaaag caccagcccg gcctatgttt gacctgaatg  2280
acccataaag agtggtatgc ctatgatgtt tgtatgtgct ctatcaataa ctaaggtgtc  2340
aactatgaac catatggtct tctgttttac ttgtttgatg tgcttggcat ggtaatccta  2400
attagcttcc tgctgtctag gtttgtagtg tgttgttttc cgtaggcata tgcatcacaa  2460
gatatcatgt aagtttcttg tcctacatat caataatgag aataaagtac ttctatgcaa  2520
tagctctgag ttaagtgttt caacaatttc tgaacttatg tttgctcagc actcagcagt  2580
catcacacga agtactctcc ttgtaactac attttcccaa agactttaaa tccccttagt  2640
tacagcaaaa aaaataaact ttgaatctat tgttttccct ctcttcggtc gatcttattg  2700
ggtacttcct ccgttattgg gtacttcctc cgtttcaggt tataagattt tctaacattg  2760
tccactttca tatagatgtt aaatgaattt agacatatgc tagaaagtct tataatctga  2820
aacgaaggta gtattattta tttgactact atagagagag gctgcatgaa gtatttcc    2878
```

SEQ ID NO: 2 moltype = DNA length = 2678

FEATURE Location/Qualifiers

source 1..2678

mol_type = genomic DNA

organism = Oryza sativa

SEQUENCE: 2

```
ctctytctyt ytcgcccaaa cccagaaacc ctcgccgccg ccgccgccgc cgccgccatc  60
acccaccatg gctacgaccg ccgcggccgc ggccgccacc ttgtccgccg ccgcgacggc  120
caagaccggc cgtaagaacc accagcgaca ccacgtcctt cccgctcgag gccgggtggg  180
ggcggcggcg gtcaggtgct cggcggtgtc cccggtcacc ccgccgtccc cggcgccgcc  240
ggccacgccg ctccggccgt gggggccggc cgagccccgc aagggcgcgg acatcctcgt  300
ggaggcgctg gagcggtgcg gcgtcagcga cgtgttcgcc tacccgggcg gcgcgtccat  360
ggagatccac caggcgctga cgcgctcccc ggtcatcacc aaccacctct tccgccacga  420
gcagggcgag gcgttcgcgg cgtccgggta cgcgcgcgcg tccggccgcg tcggggtctg  480
cgtcgccacc tccggccccg gggcaaccaa cctcgtgtcc gcgctcgccg acgcgctgct  540
cgactccgtc ccgatggtcg ccatcacggg ccaggtcccc cgccgcatga tcggcaccga  600
cgtcttccag gagacgccca tagtcgaggt cacccgctcc atcaccaagc acaattacct  660
tgtccttgat gtggaggaca tcccccgcgt catacaggaa gccttcttcc tcgcgtcctc  720
gggccgtcct ggcccggtgc tggtcgacat ccccaaggac atccagcagc agatggctgt  780
gccagtctgg gacacctcga tgaatctacc ggggtacatt gcacgcctgc ccaagccacc  840
cgcgacagaa ttgcttgagc aggtcttgcg tctggttggc gagtcacggc gcccgattct  900
ctatgtcggt ggtggctgct ctgcatctgg tgatgaattg cgccggtttg ttgagctgac  960
cggcatccca gttacaacca ctctgatggg cctcggcaat ttccccagtg atgatccgtt  1020
gtccctgcgc atgcttggga tgcatggcac ggtgtacgca aattatgcgg tggataaggc  1080
tgacctgttg cttgcatttg gcgtgcggtt tgatgatcgt gtgacaggga aaattgaggc  1140
```

-continued

```
ttttgcaagc agggccaaga ttgtgcacat tgacattgat ccagcggaga ttggaaagaa  1200
caagcaacca catgtgtcaa tttgcgcaga tgttaagctt gctttacagg gcttgaatgc  1260
tctgctagac cagagcacaa caaagacaag ttctgatttt agtgcatggc acaatgagtt  1320
ggaccagcag aagagggagt ttcctctggg gtacaagact tttggtgaag agatcccacc  1380
gcaatatgct attcaggtgc tggatgagct gacgaaaggg gaggcaatca tcgctactgg  1440
tgttggacag caccagatgt gggcggcaca atattacacc tacaagcggc cacggcagtg  1500
gctgtcttcg gctggtctgg gcgcaatggg atttgggctg cctgctgcag ctggtgcttc  1560
tgtggctaac ccaggtgtca cagttgttga tattgatggg gatggtagct tcctcatgaa  1620
cattcaggag ttggcattga tccgcattga gaacctcccg gtgaaggtga tggtgttgaa  1680
caaccaacat ttgggtatgg ttgtgcaatg ggaggatagg ttttacaagg caaatagggc  1740
gcatacatac ttgggcaacc cagaatgtga gagcgagata tatccagatt ttgtgactat  1800
tgctaaaggg ttcaatattc ctgcagtccg tgtaacaaag aagagtgaag tccgtgccgc  1860
catcaagaag atgctcgaga ccccagggcc atacttgttg gatatcatcg tcccacacca  1920
ggagcatgtg ctgcctatga tcccaagtgg gggcgcattc aaggacatga tcctggatgg  1980
tgatggcagg actatgtatt aatctataat ctgtatgttg gcaaagcacc agcccggcct  2040
atgtttgacc tgaatgaccc ataaagagtg gtatgcctat gatgtttgta tgtgctctat  2100
caataactaa ggtgtcaact atgaaccata tggtcttctg ttttacttgt ttgatgtgct  2160
tggcatggta atcctaatta gcttcctgct gtctaggttt gtagtgtgtt gttttccgta  2220
ggcatatgca tcacaagata tcatgtaagt ttcttgtcct acatatcaat aatgagaata  2280
aagtacttct atgcaatagc tctgagttaa gtgtttcaac aatttctgaa cttatgtttg  2340
ctcagcactc agcagtcatc acacgaagta ctctccttgt aactacattt tcccaaagac  2400
tttaaatccc cttagttaca gcaaaaaaaa taaactttga atctattgtt ttccctctct  2460
tcggtcgatc ttattgggta cttcctccgt tattgggtac ttcctccgtt tcaggttata  2520
agattttcta acattgtcca ctttcatata gatgttaaat gaatttagac atatgctaga  2580
aagtcttata atctgaaacg aaggtagtat tatttatttg actactatag agagaggctg  2640
catgaagtat ttccttttc tgtttagttt ttgccgtg                           2678

SEQ ID NO: 3           moltype = DNA  length = 2865
FEATURE                Location/Qualifiers
source                 1..2865
                       mol_type = genomic DNA
                       organism = Oryza sativa
SEQUENCE: 3
ctgtcatcct catcctatca actgacacgc gggcccagat cgaccccgac gtggcagtgt  60
gtcatcctat cccaccgaca tctggggccc accgtgacgt ggcccacacg atcccatccg  120
agccacacat cgcctcacgc tgcgtcaccg cgcgcggaca aaacacccac acccccacac  180
tctccacccc tctctctctc tctcgcccaa acccagaaac cctcgccgcc gccgccgccg  240
ccgccgccat cacccaccat ggctacgacc gccgcggccg cggccgccac cttgtccgcc  300
gccgcgacgg ccaagaccgg ccgtaagaac caccagcgac accacgtcct tcccgctcga  360
ggccgggtgg gggcggcggc ggtcaggtgc tcggcggtgt ccccggtcac cccgccgtcc  420
ccggcgccgc cggccacgcc gctccggccg tgggggccgg ccgagccccg caagggcgcg  480
gacatcctcg tggaggcgct ggagcggtgc ggcgtcagcg acgtgttcgc ctacccgggc  540
ggcgcgtcca tggagatcca ccaggcgctg acgcgctccc cggtcatcac caaccacctc  600
ttccgccacg agcagggcga ggcgttcgcg gcgtccgggt acgcgcgcgc gtccggccgc  660
gtcggggtct gcgtcgccac ctccggcccc ggggcaacca acctcgtgtc cgcgctcgcc  720
gacgcgctgc tcgactccgt cccgatggtc gccatcacgg gccaggtccc ccgccgcatg  780
atcggcaccg acgccttcca ggagacgccc atagtcgagg tcacccgctc catcaccaag  840
cacaattacc ttgtccttga tgtggaggac atcccccgcg tcatacagga agccttcttc  900
ctcgcgtcct cgggccgtcc tggcccggtg ctggtcgaca tccccaagga catccagcag  960
cagatggctg tgccagtctg ggacacctcg atgaatctac cggggtacat tgcacgcctg  1020
cccaagccac ccgcgacaga attgcttgag caggtcttgc gtctggttgg cgagtcacgg  1080
cgcccgattc tctatgtcgg tggtggctgc tctgcatctg gtgatgaatt gcgccggttt  1140
gttgagctga ccggcatccc agttacaacc actctgatgg gcctcggcaa tttccccagt  1200
gatgatccgt tgtccctgcg catgcttggg atgcatggca cggtgtacgc aaattatgcg  1260
gtggataagg ctgacctgtt gcttgcattt ggcgtgcggt ttgatgatcg tgtgacaggg  1320
aaaattgagg cttttgcaag cagggccaag attgtgcaca ttgacattga tccagcggag  1380
attggaaaga acaagcaacc acatgtgtca atttgcgcag atgttaagct tgctttacag  1440
ggcttgaatg ctctgctaga ccagagcaca acaaagacaa gttctgattt tagtgcatgg  1500
cacaatgagt tggaccagca gaagagggag tttcctctgg ggtacaagac ttttggtgaa  1560
gagatcccac cgcaatatgc tattcaggtg ctggatgagc tgacgaaagg ggaggcaatc  1620
atcgctactg gtgttggaca gcaccagatg tgggcggcac aatattacac ctacaagcgg  1680
ccacggcagt ggctgtcttc ggctggtctg ggcgcaatgg gatttgggct gcctgctgca  1740
gctggtgctt ctgtggctaa cccaggtgtc acagttgttg atattgatgg ggatggtagc  1800
ttcctcatga acattcagga gttggcattg atccgcattg agaacctccc ggtgaaggtg  1860
atggtgttga acaaccaaca tttgggtatg gttgtgcaat gggaggatag gttttacaag  1920
gcaaatagggc cgcatacata cttgggcaac ccagaatgtg agagcgagat atatccagat  1980
tttgtgacta ttgctaaagg gttcaatatt cctgcagtcc gtgtaacaaa gaagagtgaa  2040
gtccgtgccg ccatcaagaa gatgctcgag accccagggc catacttgtt ggatatcatc  2100
gtcccacacc aggagcatgt gctgcctatg atcccaagtg ggggcgcatt caaggacatg  2160
atcctggatg gtgatggcag gactatgtat taatctataa tctgtatgtt ggcaaagcac  2220
cagcccggcc tatgtttgac ctgaatgacc cataaagagt ggtatgccta tgatgtttgt  2280
atgtgctcta tcaataacta aggtgtcaac tatgaaccat atggtcttct gttttacttg  2340
tttgatgtgc ttggcatggt aatcctaatt agcttcctgc tgtctaggtt tgtagtgtgt  2400
tgttttccgt aggcatatgc atcacaagat atcatgtaag tttcttgtcc tacatatcaa  2460
taatgagaat aaagtacttc tatgcaatag ctctgagtta agtgtttcaa caatttctga  2520
acttatgttt gctcagcact cagcagtcat cacacgaagt actctccttg taactacatt  2580
```

-continued

```
ttcccaaaga ctttaaatcc ccttagttac agcaaaaaaa ataaactttg aatctattgt  2640
tttccctctc ttcggtcgat cttattgggt acttcctccg ttattgggta cttcctccgt  2700
ttcaggttat aagattttct aacattgtcc actttcatat agatgttaaa tgaatttaga  2760
catatgctag aaagtcttat aatctgaaac gaaggtagta ttatttattt gactactata  2820
gagagaggct gcatgaagta tttccttttt ctgtttagtt ttgcc                  2865

SEQ ID NO: 4           moltype = DNA  length = 2877
FEATURE                Location/Qualifiers
source                 1..2877
                       mol_type = genomic DNA
                       organism = Oryza sativa
SEQUENCE: 4
agttytgtac cctccacccg ttattgggas ggatccacct gtcatcctca tcctatcaac  60
tgacacgcgg gcccagatcg accccgacgt ggcagtgtgt catcctatcc caccgacatc  120
tggggcccac cgtgacgtgg cccacacgat cccatccgag ccacacatcg cctcacgctg  180
cgtcaccgcg cgcggacaaa acacccacac ccccacactc tccacccctc tctctctctc  240
tcgcccaaac ccagaaaccc tcgccgccgc cgccgccgcc gccgccatca cccaccatgg  300
ctacgaccgc cgcggccgcg gccgccacct tgtccgccgc cgcgacggcc aagaccggcc  360
gtaagaacca ccagcgacac cacgtccttc ccgctcgagg ccgggtgggg gcggcggcgg  420
tcaggtgctc ggcggtgtcc ccggtcaccc cgccgtcccc ggcgccgccg gccacgccgc  480
tccggccgtg ggggccggcc gagccccgca agggcgcgga catcctcgtg gaggcgctgg  540
agcggtgcgg cgtcagcgac gtgttcgcct acccgggcgg cgcgtccatg gagatccacc  600
aggcgctgac gcgctccccg gtcatcacca accacctctt ccgccacgag cagggcgagg  660
cgttcgcggc gtccgggtac gcgcgcgcgt ccggccgcgt cggggtctgc gtcgccacct  720
ccggccccgg ggcaaccaac ctcgtgtccg cgctcgccga cgcgctgctc gactccgtcc  780
cgatggtcgc catcacgggc caggtccccc gccgcatgat cggcaccgac gccttccagg  840
agacgcccat agtcgaggtc acccgctcca tcaccaagca caattacctt gtccttgatg  900
tggaggacat cccccgcgtc atacaggaag ccttcttcct cgcgtcctcg ggccgtcctg  960
gcccggtgct ggtcgacatc cccaaggaca tccagcagca gatggctgtg ccagtctggg  1020
acacctcgat gaatctaccg gggtacattg cacgcctgcc caagccaccc gcgacagaat  1080
tgcttgagca ggtcttgcgt ctggttggcg agtcacggcg cccgattctc tatgtcggtg  1140
gtggctgctc tgcatctggt gatgaattgc gccggtttgt tgagctgacc ggcatcccag  1200
ttacaaccac tctgatgggc ctcggcaatt tccccagtga tgatccgttg tccctgcgca  1260
tgcttgggat gcatggcacg gtgtacgcaa attatgcggt ggataaggct gacctgttgc  1320
ttgcatttgg cgtgcggttt gatgatcgtg tgacagggaa aattgaggct tttgcaagca  1380
gggccaagat tgtgcacatt gacattgatc cagcggagat tggaaagaac aagcaaccac  1440
atgtgtcaat ttgcgcagat gttaagcttg ctttacaggg cttgaatgct ctgctagacc  1500
agagcacaac aaagacaagt tctgatttta gtgcatggca caatgagttg gaccagcaga  1560
agagggagtt tcctctgggg tacaagactt ttggtgaaga gatcccaccg caatatgcta  1620
ttcaggtgct ggatgagctg acgaaagggg aggcaatcat cgctactggt gttggacagc  1680
accagatgtg ggcggcacaa tattacacct acaagcggcc acggcagtgg ctgtcttcgg  1740
ctggtctggg cgcaatggga tttgggctgc ctgctgcagc tggtgcttct gtggctaacc  1800
caggtgtcac agttgttgat attgatgggg atggtagctt cctcatgaac attcaggagt  1860
tggcattgat ccgcattgag aacctcccgg tgaaggtgat ggtgttgaac aaccaacatt  1920
tgggtatggt tgtgcaatgg gaggataggt tttacaaggc aaatagggcg catacatact  1980
tgggcaaccc agaatgtgag agcgagatat atccagattt tgtgactatt gctaaagggt  2040
tcaatattcc tgcagtccgt gtaacaaaga agagtgaagt ccgtgccgcc atcaagaaga  2100
tgctcgagac cccagggcca tacttgttgg atatcatcgt cccacaccag gagcatgtgc  2160
tgcctatgat cccaagtgag ggcgcattca aggacatgat cctggatggt gatggcagga  2220
ctatgtatta atctataatc tgtatgttgg caaagcacca gcccggccta tgtttgacct  2280
gaatgaccca taaagagtgg tatgcctatg atgtttgtat gtgctctatc aataactaag  2340
gtgtcaacta tgaaccgtat ggtcttctgt tttacttgtt tgatgtgctt ggcatggtaa  2400
tcctaattag cttcctgctg tctaggtttg tagtgtgttg ttttccgtag gcatatgcat  2460
cacaagatat catgtaagtt tcttgtccta catatcaata atgagaataa agtacttcta  2520
tgcaatagct ctgagttaag tgtttcaaca atttctgaac ttatgtttgc tcagcactca  2580
gcagtcatca cacgaagtac tctccttgta actacatttt cccaaagact ttaaatcccc  2640
ttagttacag caaaaaaaat aaactttgaa tctattgttt tccctctctt cggtcgatct  2700
tattgggtac ttcctccgtt attgggtact tcctccgttt caggttataa gattttctaa  2760
cattgtccac tttcatatag atgttaaatg aatttagaca tatgctagaa agtcttataa  2820
tctgaaacga aggtagtatt atttatttga ctactataga gagaggctgc atgaagt     2877

SEQ ID NO: 5           moltype = AA  length = 644
FEATURE                Location/Qualifiers
source                 1..644
                       mol_type = protein
                       organism = Oryza sativa
SEQUENCE: 5
MATTAAAAAA TLSAAATAKT GRKNHQRHHV LPARGRVGAA AVRCSAVSPV TPPSPAPPAT  60
PLRPWGPAEP RKGADILVEA LERCGVSDVF AYPGGASMEI HQALTRSPVI TNHLFRHEQG  120
EAFAASGYAR ASGRVGVCVA TSGPGATNLV SALADALLDS VPMVAITGQV PRRMIGTDVF  180
QETPIVEVTR SITKHNYLVL DVEDIPRVIQ EAFFLASSGR PGPVLVDIPK DIQQQMAVPV  240
WDTSMNLPGY IARLPKPPAT ELLEQVLRLV GESRRPILYV GGGCSASGDE LRRFVELTGI  300
PVTTTLMGLG NFPSDDPLSL RMLGMHGTVY ANYAVDKADL LLAFGVRFDD RVTGKIEAFA  360
SRAKIVHIDI DPAEIGKNKQ PHVSICADVK LALQGLNALL DQSTTKTSSD FSAWHNELDQ  420
QKREFPLGYK TFGEEIPPQY AIQVLDELTK GEAIIATGVG QHQMWAAQYY TYKRPRQWLS  480
SAGLGAMGFG LPAAAGASVA NPGVTVVDID GDGSFLMNIQ ELALIRIENL PVKVMVLNNQ  540
```

-continued

```
HLGMVVQWED RFYKANRAHT YLGNPECESE IYPDFVTIAK GFNIPAVRVT KKSEVRAAIK  600
KMLETPGPYL LDIIVPHQEH VLPMIPSGGA FKDMILDGDG RTMY                  644

SEQ ID NO: 6          moltype = AA  length = 644
FEATURE               Location/Qualifiers
source                1..644
                      mol_type = protein
                      organism = Oryza sativa
SEQUENCE: 6
MATTAAAAAA TLSAAATAKT GRKNHQRHHV LPARGRVGAA AVRCSAVSPV TPPSPAPPAT  60
PLRPWGPAEP RKGADILVEA LERCGVSDVF AYPGGASMEI HQALTRSPVI TNHLFRHEQG  120
EAFAASGYAR ASGRVGVCVA TSGPGATNLV SALADALLDS VPMVAITGQV PRRMIGTDAF  180
QETPIVEVTR SITKHNYLVL DVEDIPRVIQ EAFFLASSGR PGPVLVDIPK DIQQQMAVPV  240
WDTSMNLPGY IARLPKPPAT ELLEQVLRLV GESRRPILYV GGGCSASGDE LRRFVELTGI  300
PVTTTLMGLG NFPSDDPLSL RMLGMHGTVY ANYAVDKADL LLAFGVRFDD RVTGKIEAFA  360
SRAKIVHIDI DPAEIGKNKQ PHVSICADVK LALQGLNALL DQSTTKTSSD FSAWHNELDQ  420
QKREFPLGYK TFGEEIPPQY AIQVLDELTK GEAIIATGVG QHQMWAAQYY TYKRPRQWLS  480
SAGLGAMGFG LPAAAGASVA NPGVTVVDID GDGSFLMNIQ ELALIRIENL PVKVMVLNNQ  540
HLGMVVQWED RFYKANRAHT YLGNPECESE IYPDFVTIAK GFNIPAVRVT KKSEVRAAIK  600
KMLETPGPYL LDIIVPHQEH VLPMIPNGGA FKDMILDGDG RTMY                  644

SEQ ID NO: 7          moltype = AA  length = 644
FEATURE               Location/Qualifiers
source                1..644
                      mol_type = protein
                      organism = Oryza sativa
SEQUENCE: 7
MATTAAAAAA TLSAAATAKT GRKNHQRHHV LPARGRVGAA AVRCSAVSPV TPPSPAPPAT  60
PLRPWGPAEP RKGADILVEA LERCGVSDVF AYPGGASMEI HQALTRSPVI TNHLFRHEQG  120
EAFAASGYAR ASGRVGVCVA TSGPGATNLV SALADALLDS VPMVAITGQV PRRMIGTDAF  180
QETPIVEVTR SITKHNYLVL DVEDIPRVIQ EAFFLASSGR PGPVLVDIPK DIQQQMAVPV  240
WDTSMNLPGY IARLPKPPAT ELLEQVLRLV GESRRPILYV GGGCSASGDE LRRFVELTGI  300
PVTTTLMGLG NFPSDDPLSL RMLGMHGTVY ANYAVDKADL LLAFGVRFDD RVTGKIEAFA  360
SRAKIVHIDI DPAEIGKNKQ PHVSICADVK LALQGLNALL DQSTTKTSSD FSAWHNELDQ  420
QKREFPLGYK TFGEEIPPQY AIQVLDELTK GEAIIATGVG QHQMWAAQYY TYKRPRQWLS  480
SAGLGAMGFG LPAAAGASVA NPGVTVVDID GDGSFLMNIQ ELALIRIENL PVKVMVLNNQ  540
HLGMVVQWED RFYKANRAHT YLGNPECESE IYPDFVTIAK GFNIPAVRVT KKSEVRAAIK  600
KMLETPGPYL LDIIVPHQEH VLPMIPSEGA FKDMILDGDG RTMY                  644

SEQ ID NO: 8          moltype = AA  length = 644
FEATURE               Location/Qualifiers
source                1..644
                      mol_type = protein
                      organism = Oryza sativa
SEQUENCE: 8
MATTAAAAAA TLSAAATAKT GRKNHQRHHV LPARGRVGAA AVRCSAVSPV TPPSPAPPAT  60
PLRPWGPAEP RKGADILVEA LERCGVSDVF AYPGGASMEI HQALTRSPVI TNHLFRHEQG  120
EAFAASGYAR ASGRVGVCVA TSGPGATNLV SALADALLDS VPMVAITGQV PRRMIGTDAF  180
QETPIVEVTR SITKHNYLVL DVEDIPRVIQ EAFFLASSGR PGPVLVDIPK DIQQQMAVPV  240
WDTSMNLPGY IARLPKPPAT ELLEQVLRLV GESRRPILYV GGGCSASGDE LRRFVELTGI  300
PVTTTLMGLG NFPSDDPLSL RMLGMHGTVY ANYAVDKADL LLAFGVRFDD RVTGKIEAFA  360
SRAKIVHIDI DPAEIGKNKQ PHVSICADVK LALQGLNALL DQSTTKTSSD FSAWHNELDQ  420
QKREFPLGYK TFGEEIPPQY AIQVLDELTK GEAIIATGVG QHQMWAAQYY TYKRPRQWLS  480
SAGLGAMGFG LPAAAGASVA NPGVTVVDID GDGSFLMNIQ ELALIRIENL PVKVMVLNNQ  540
HLGMVVQWED RFYKANRAHT YLGNPECESE IYPDFVTIAK GFNIPAVRVT KKSEVRAAIK  600
KMLETPGPYL LDIIVPHQEH VLPMIPNGGA FKDMILDGDG RTMY                  644

SEQ ID NO: 9          moltype = AA  length = 644
FEATURE               Location/Qualifiers
source                1..644
                      mol_type = protein
                      organism = Oryza sativa
SEQUENCE: 9
MATTAAAAAA TLSAAATAKT GRKNHQRHHV LPARGRVGAA AVRCSAVSPV TPPSPAPPAT  60
PLRPWGPAEP RKGADILVEA LERCGVSDVF AYPGGASMEI HQALTRSPVI TNHLFRHEQG  120
EAFAASGYAR ASGRVGVCVA TSGPGATNLV SALADALLDS VPMVAITGQV PRRMIGTDAF  180
QETPIVEVTR SITKHNYLVL DVEDIPRVIQ EAFFLASSGR PGPVLVDIPK DIQQQMAVPV  240
WDTSMNLPGY IARLPKPPAT ELLEQVLRLV GESRRPILYV GGGCSASGDE LRRFVELTGI  300
PVTTTLMGLG NFPSDDPLSL RMLGMHGTVY ANYAVDKADL LLAFGVRFDD RVTGKIEAFA  360
SRAKIVHIDI DPAEIGKNKQ PHVSICADVK LALQGLNALL DQSTTKTSSD FSAWHNELDQ  420
QKREFPLGYK TFGEEIPPQY AIQVLDELTK GEAIIATGVG QHQMWAAQYY TYKRPRQWLS  480
SAGLGAMGFG LPAAAGASVA NPGVTVVDID GDGSFLMNIQ ELALIRIENL PVKVMVLNNQ  540
HLGMVVQWED RFYKANRAHT YLGNPECESE IYPDFVTIAK GFNIPAVRVT KKSEVRAAIK  600
KMLETPGPYL LDIIVPHQEH VLPMIPSGGA FKDMILDGDG RTMY                  644

SEQ ID NO: 10         moltype = AA  length = 644
```

-continued

```
FEATURE                 Location/Qualifiers
source                  1..644
                        mol_type = protein
                        organism = Oryza sativa
SEQUENCE: 10
MATTAAAAAA TLSAAATAKT GRKNHQRHHV LPARGRVGAA AVRCSAVSPV TPPSPAPPAT  60
PLRPWGPAEP RKGADILVEA LERCGVSDVF AYPGGASMEI HQALTRSPVI TNHLFRHEQG  120
EAFAASGYAR ASGRVGVCVA TSGPGATNLV SALADALLDS VPMVAITGQV PRRMIGTDAF  180
QETPIVEVTR SITKHNYLVL DVEDIPRVIQ EAFFLASSGR PGPVLVDIPK DIQQQMAVPV  240
WDTSMNLPGY IARLPKPPAT ELLEQVLRLV GESRRPILYV GGGCSASGDE LRRFVELTGI  300
PVTTTLMGLG NFPSDDPLSL RMLGMHGTVY ANYAVDKADL LLAFGVRFDD RVTGKIEAFA  360
SRAKIVHIDI DPAEIGKNKQ PHVSICADVK LALQGLNALL DQSTTKTSSD FSAWHNELDQ  420
QKREFPLGYK TFGEEIPPQY AIQVLDELTK GEAIIATGVG QHQMWAAQYY TYKRPRQWLS  480
SAGLGAMGFG LPAAAGASVA NPGVTVVDID GDGSFLMNIQ ELALIRIENL PVKVMVLNNQ  540
HLGMVVQWED RFYKANRAHT YLGNPECESE IYPDFVTIAK GFNIPAVRVT KKSEVRAAIK  600
KMLETPGPYL LDIIVPHQEH VLPMIPSGGA FKDMILDGDG RTMY                    644

SEQ ID NO: 11           moltype = AA  length = 644
FEATURE                 Location/Qualifiers
source                  1..644
                        mol_type = protein
                        organism = Oryza sativa
SEQUENCE: 11
MATTAAAAAA TLSAAATAKT GRKNHQRHHV LPARGRVGAA AVRCSAVSPV TPPSPAPPAT  60
PLRPWGPAEP RKGADILVEA LERCGVSDVF AYPGGASMEI HQALTRSPVI TNHLFRHEQG  120
EAFAASGYAR ASGRVGVCVA TSGPGATNLV SALADALLDS VPMVAITGQV PRRMIGTDAF  180
QETPIVEVTR SITKHNYLVL DVEDIPRVIQ EAFFLASSGR PGPVLVDIPK DIQQQMAVPV  240
WDTSMNLPGY IARLPKPPAT ELLEQVLRLV GESRRPILYV GGGCSASGDE LRRFVELTGI  300
PVTTTLMGLG NFPSDDPLSL RMLGMHGTVY ANYAVDKADL LLAFGVRFDD RVTGKIEAFA  360
SRAKIVHIDI DPAEIGKNKQ PHVSICADVK LALQGLNALL DQSTTKTSSD FSAWHNELDQ  420
QKREFPLGYK TFGEEIPPQY AIQVLDELTK GEAIIATGVG QHQMWAAQYY TYKRPRQWLS  480
SAGLGAMGFG LPAAAGASVA NPGVTVVDID GDGSFLMNIQ ELALIRIENL PVKVMVLNNQ  540
HLGMVVQWED RFYKANRAHT YLGNPECESE IYPDFVTIAK GFNIPAVRVT KKSEVRAAIK  600
KMLETPGPYL LDIIVPHQEH VLPMIPSGGA FKDMILDGDG RTMY                    644

SEQ ID NO: 12           moltype = AA  length = 644
FEATURE                 Location/Qualifiers
source                  1..644
                        mol_type = protein
                        organism = Oryza sativa
SEQUENCE: 12
MATTAAAAAA ALSAAATAKT GRKNHQRHHV LPARGRVGAA AVRCSAVSPV TPPSPAPPAT  60
PLRPWGPAEP RKGADILVEA LERCGVSDVF AYPGGASMEI HQALTRSPVI TNHLFRHEQG  120
EAFAASGYAR ASGRVGVCVA TSGPGATNLV SALADALLDS VPMVAITGQV PRRMIGTDAF  180
QETPIVEVTR SITKHNYLVL DVEDIPRVIQ EAFFLASSGR PGPVLVDIPK DIQQQMAVPV  240
WDTSMNLPGY IARLPKPPAT ELLEQVLRLV GESRRPILYV GGGCSASGDE LRWFVELTGI  300
PVTTTLMGLG NFPSDDPLSL RMLGMHGTVY ANYAVDKADL LLAFGVRFDD RVTGKIEAFA  360
SRAKIVHIDI DPAEIGKNKQ PHVSICADVK LALQGLNALL QQSTTKTSSD FSAWHNELDQ  420
QKREFPLGYK TFGEEIPPQY AIQVLDELTK GEAIIATGVG QHQMWAAQYY TYKRPRQWLS  480
SAGLGAMGFG LPAAAGASVA NPGVTVVDID GDGSFLMNIQ ELALIRIENL PVKVMVLNNQ  540
HLGMVVQWED RFYKANRAHT YLGNPECESE IYPDFVTIAK GFNIPAVRVT KKSEVRAAIK  600
KMLETPGPYL LDIIVPHQEH VLPMIPSGGA FKDMILDGDG RTVY                    644

SEQ ID NO: 13           moltype = AA  length = 669
FEATURE                 Location/Qualifiers
source                  1..669
                        mol_type = protein
                        organism = Arabidopsis thaliana
SEQUENCE: 13
MAAATTTTTT SSSISFSTKP SPSSSKSPLP ISRFSLPFSL NPNKSSSSSR RRGIKSSSPS  60
SISAVLNTTT NVTTTPSPTK PTKPETFISR FAPDQPRKGA DILVEALERQ GVETVFAYPG  120
GASMEIHQAL TRSSSIRNVL PRHEQGGVFA AEGYARSSGK PGICIATSGP GATNLVSGLA  180
DALLDSVPLV AITGQVPRRM IGTDAFQETP IVEVTRSITK HNYLVMDVED IPRIIEEAFF  240
LATSGRPGPV LVDVPKDIQQ QLAIPNWEQA MRLPGYMSRM PKPPEDSHLE QIVRLISESK  300
KPVLYVGGGC LNSSDELGRF VELTGIPVAS TLMGLGSYPC DDELSLHMLG MHGTVYANYA  360
VEHSDLLLAF GVRFDDRVTG KLEAFASRAK IVHIDIDSAE IGKNKTPHVS VCGDVKLALQ  420
GMNKVLENRA EELKLDFGVW RNELNVQKQK FPLSFKTFGE AIPPQYAIKV LDELTDGKAI  480
ISTGVGQHQM WAAQFYNYKK PRQWLSSGGL GAMGFGLPAA IGASVANPDA IVVDIDGDGS  540
FIMNVQELAT IRVENLPVKV LLLNNQHLGM VMQWEDRFYK ANRAHTFLGD PAQEDEIFPN  600
MLLFAAACGI PAARVTKKAD LREAIQTMLD TPGPYLLDVI CPHQEHVLPM IPSGGTFNDV  660
ITEGDGRIK                                                           669
```

1-12. (canceled)

13. A method to protect rice in a field, the method comprising:

a. obtaining a rice plant wherein the rice plant is resistant to AHAS/ALS inhibitors at dosages levels that cause more plant injury to rice plants without a mutation in a gene encoding a substitution of amino acids A205V in an AHAS/ALS enzyme, compared to rice plants with the mutation; and

b. contacting the rice plant with at least one AHAS/ALS inhibitors at dosage levels to which the rice plant is resistant.

14. The method of claim **13**, wherein the AHAS/ALS inhibitors cause 10% or less plant injury to the rice plant.

15. The method of claim **13**, wherein the AHAS/ALS inhibitors comprise at least one imidazolinone herbicides.

16. The method of claim **14**, wherein the imidazolinone herbicides are selected from the group consisting of imazethapyr, imazamox, imazapic, imazamethabenz, imazaquin, and combinations thereof.

17. The method of claim **13**, wherein the AHAS/ALS inhibitors are administered at a rice development stage from 2-leaf stage, 3-leaf stage, and 4-leaf stage.

18. The method of claim **13**, wherein the AHAS/ALS inhibitors are administered at two times manufacturer recommended dosage.

19. A rice plant its progeny comprising in their genome, a gene encoding an amino acid substitution A205V in an AHAS/ALS enzyme, wherein the rice plant exhibits increased tolerance to AHAS/ALS inhibiting herbicides compared to a rice plant with only a single copy of the gene.

20. A method of producing a rice seed, the method comprising:

a. crossing the rice plant of claim **19** with a rice plant of a different genetic background not having an A205V amino acid substitution in the AHAS/ALS enzyme, and

b. harvesting resultant hybrid rice seed for use in breeding AHAS/ALS inhibiting herbicide tolerant rice.

21. An herbicide resistant rice plant from a seed produced by the method of claim **20**.

* * * * *