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(12) **United States Patent**
Umemoto et al.

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(45) **Date of Patent:** Aug. 19, 2025

(54) **PLANT HAVING ENHANCED RESISTANCE AGAINST COLORADO POTATO BEETLE AND METHOD FOR PRODUCING SAME, AND METHOD FOR EVALUATING RESISTANCE AGAINST COLORADO POTATO BEETLE IN PLANT**

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(73) Assignees: **KANEKA CORPORATION**, Osaka (JP); **NATIONAL UNIVERSITY CORPORATION KOBE UNIVERSITY**, Hyogo (JP); **NATIONAL AGRICULTURE AND FOOD RESEARCH ORGANIZATION**, Ibaraki (JP); **RIKEN**, Saitama (JP)

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(51) **Int. Cl.**

C12N 15/82 (2006.01)
A01H 1/00 (2006.01)
A01H 6/82 (2018.01)
C12N 15/11 (2006.01)

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

CPC *C12N 15/8286* (2013.01); *A01H 1/127* (2021.01); *A01H 6/827* (2018.05); *C12N 15/11* (2013.01)

(58) **Field of Classification Search**

None

See application file for complete search history.

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Primary Examiner — Matthew R Keogh

(74) Attorney, Agent, or Firm — IPUSA, PLLC

(57) **ABSTRACT**

The spirosolane skeleton 23-position hydroxylase gene and the spirosolane skeleton 23-position acetyltransferase gene derived from *S. chacoense*, *S. tuberosum*, and *S. lycopersicum* are found to be involved with the biosynthesis of leptine, which achieves resistance against Colorado potato beetle.

6 Claims, 19 Drawing Sheets

Specification includes a Sequence Listing.

FIG.1

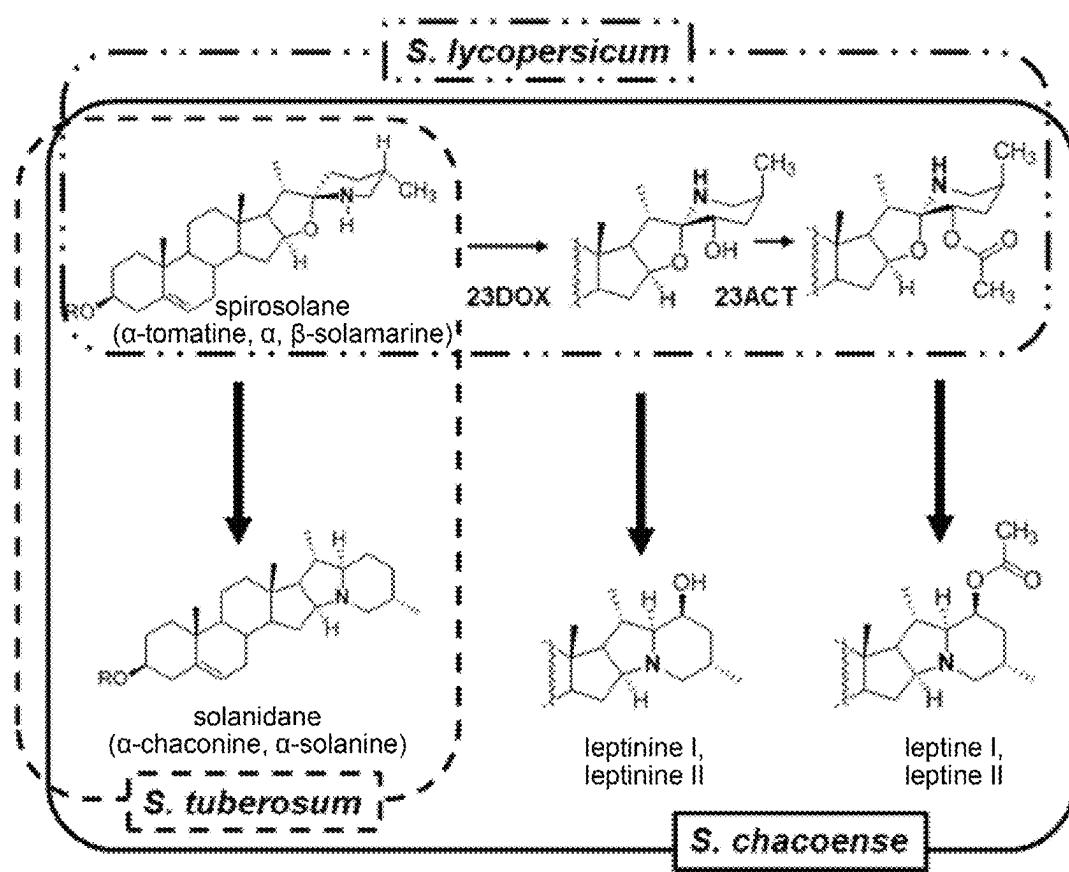


FIG.2

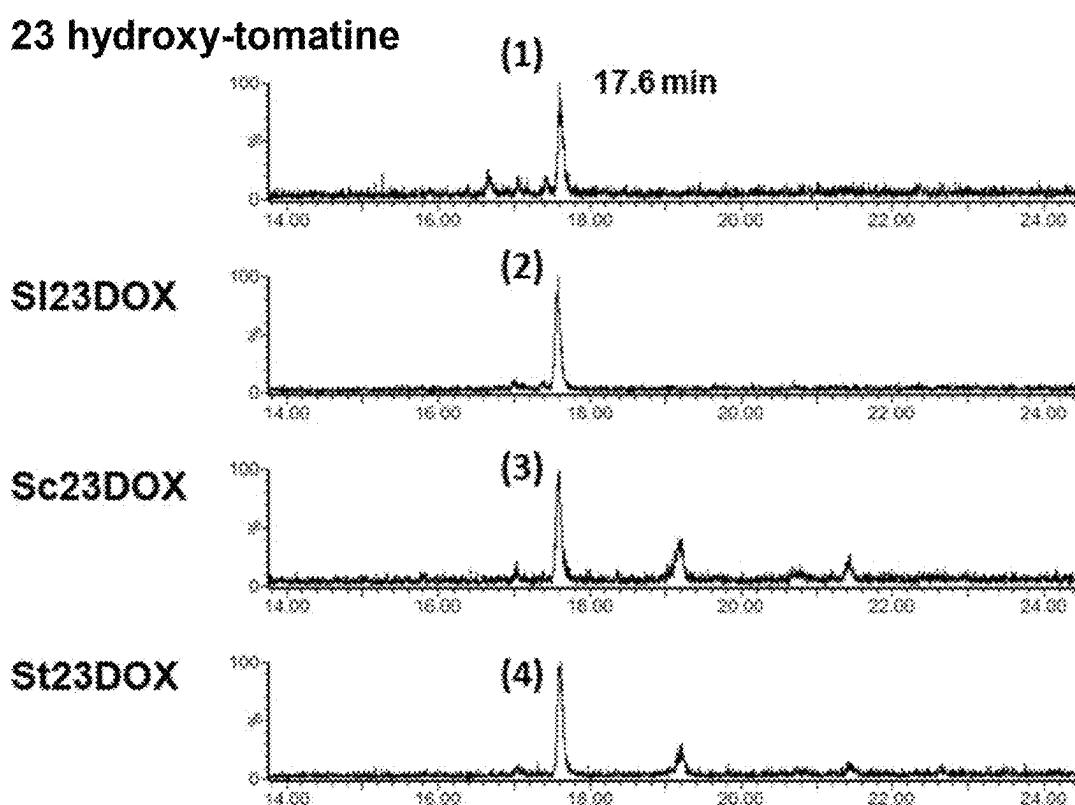


FIG.3

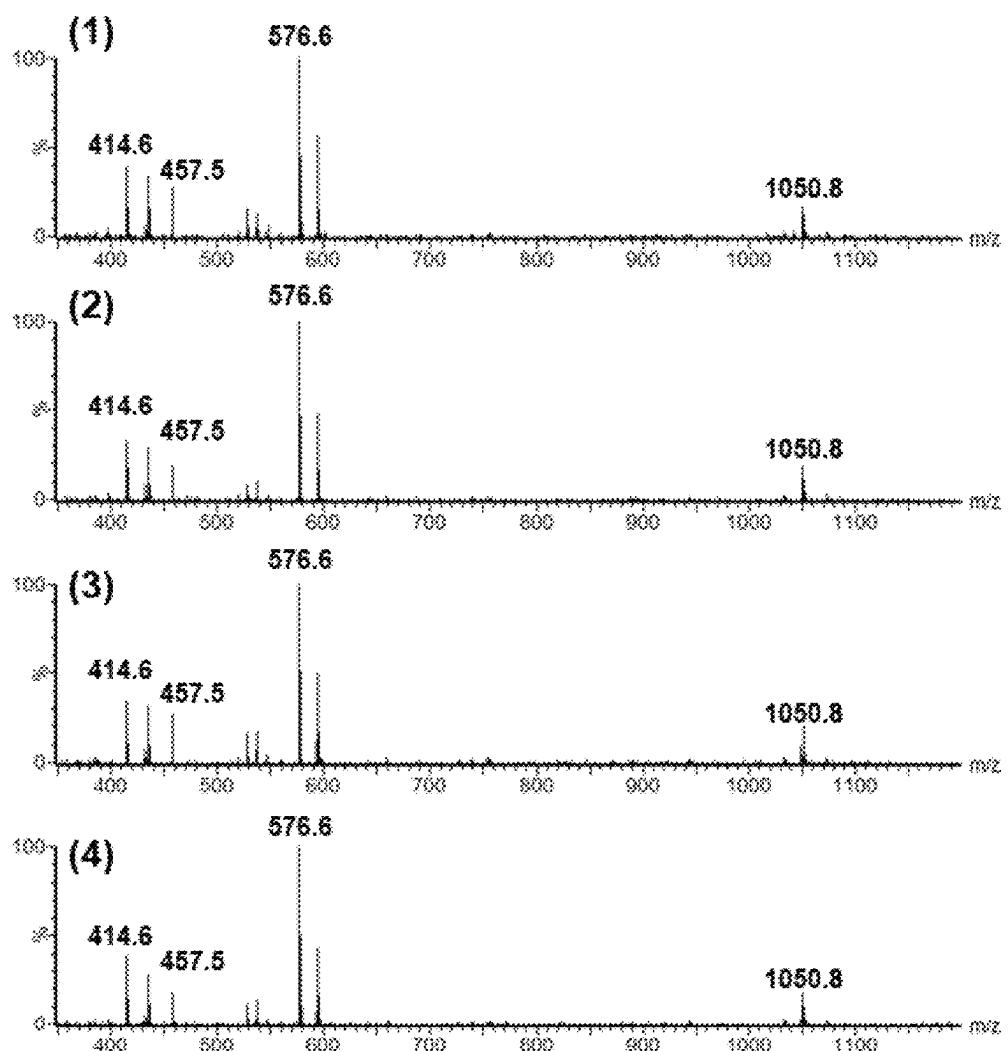


FIG.4

23 hydroxy-tomatine

+negative control

23 hydroxy-tomatine

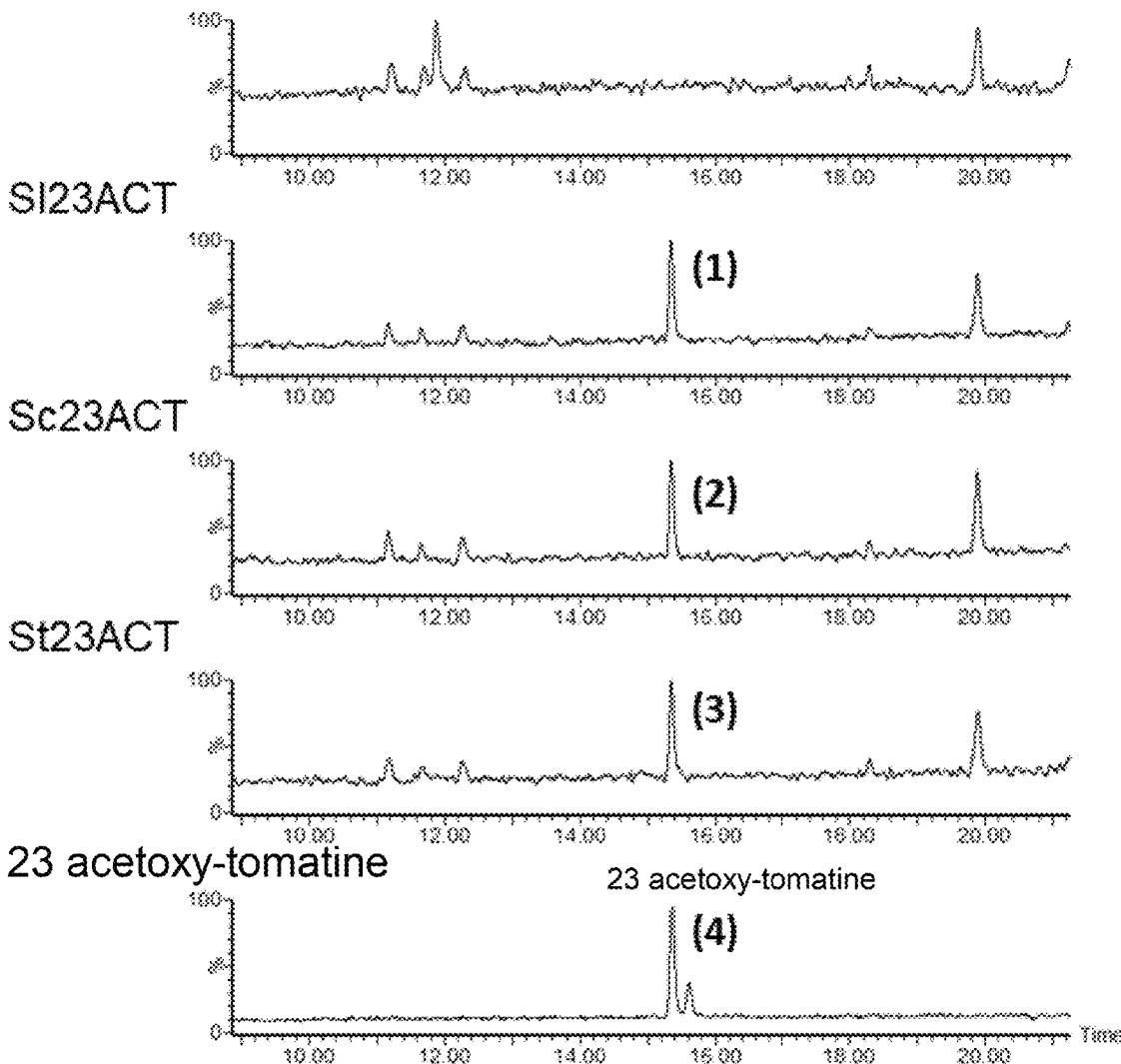


FIG.5

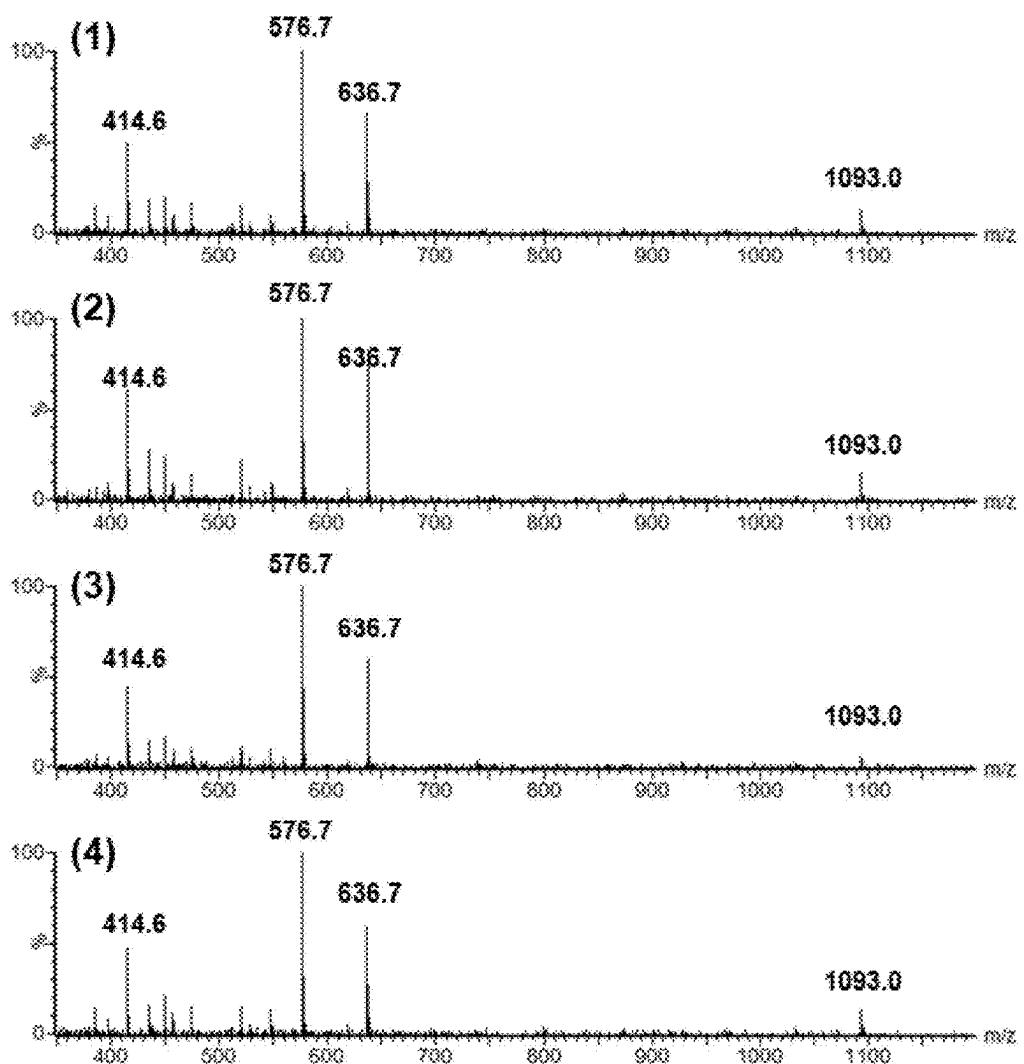


FIG.6

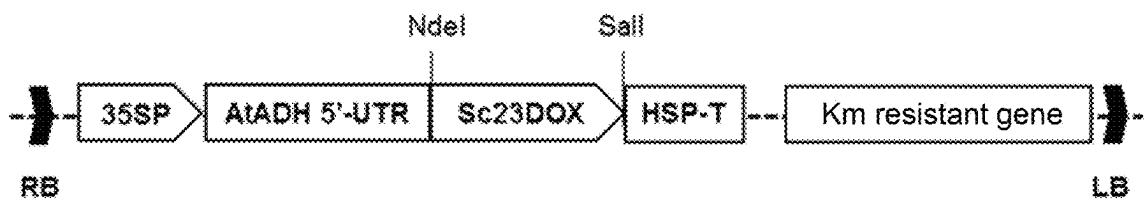
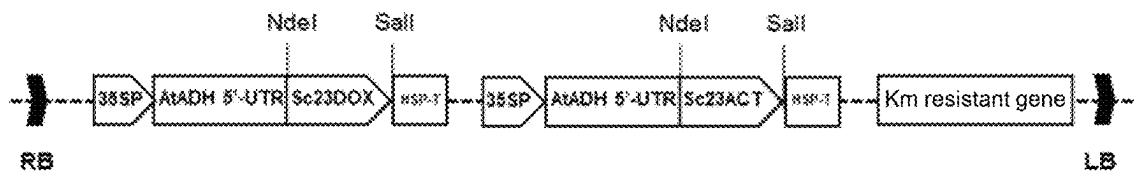
pRI201_Sc23DOX**pBIN+201_Sc23DOX_Sc23ACT**

FIG.7

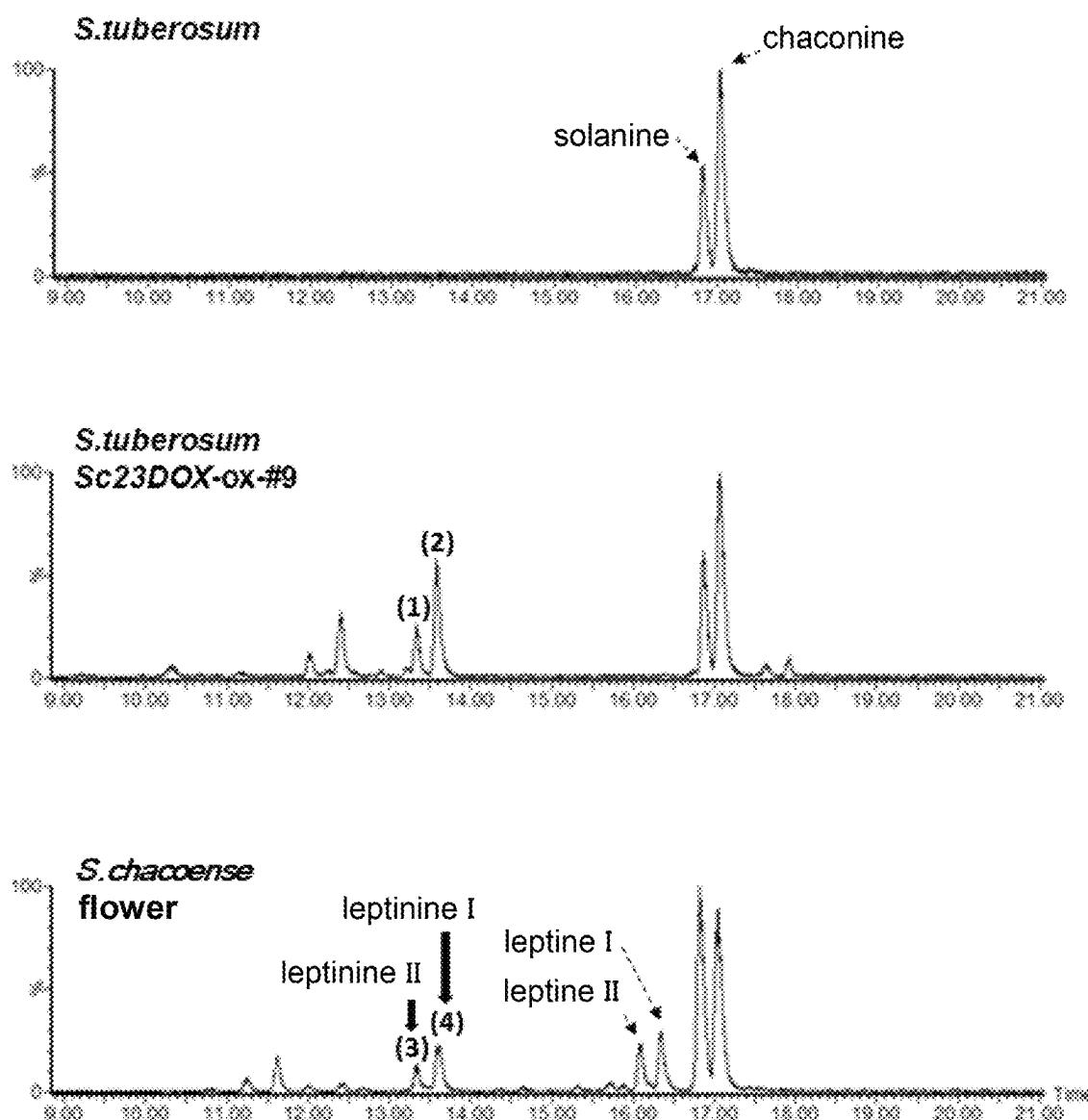


FIG.8

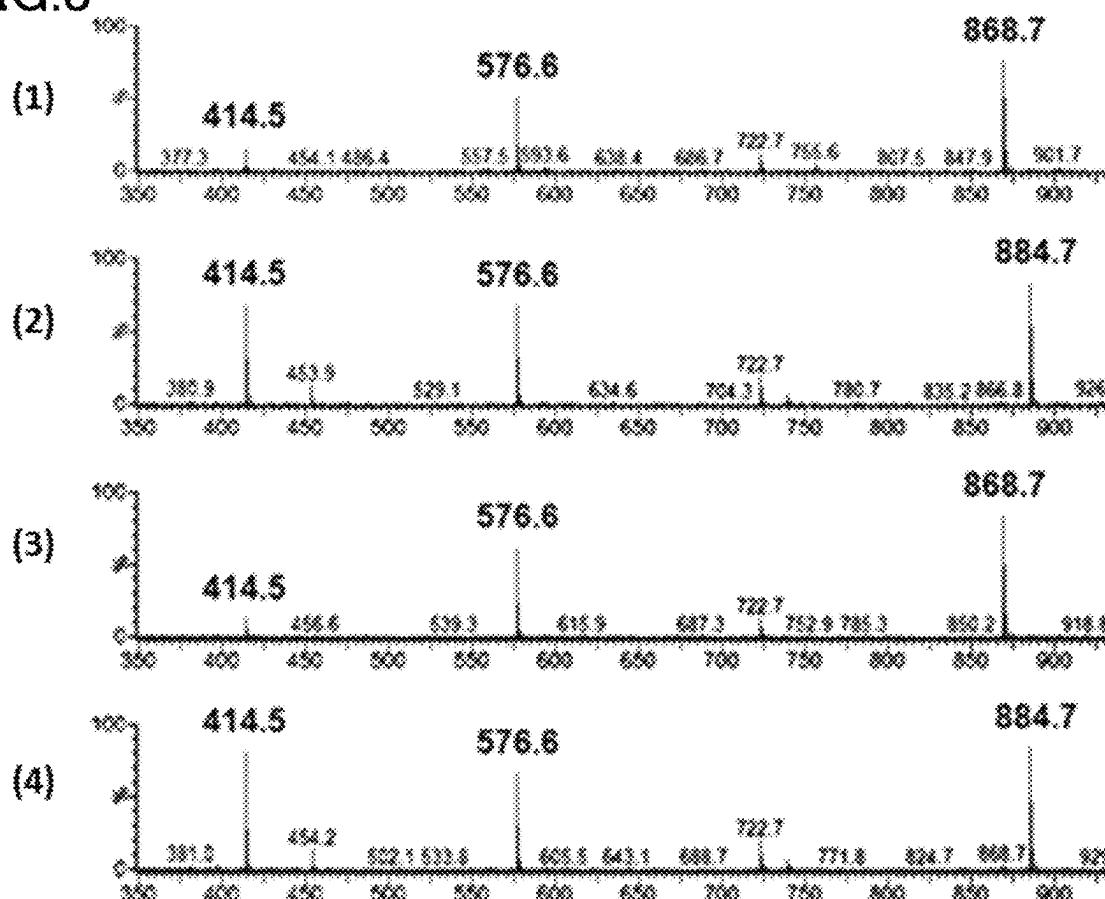


FIG.9

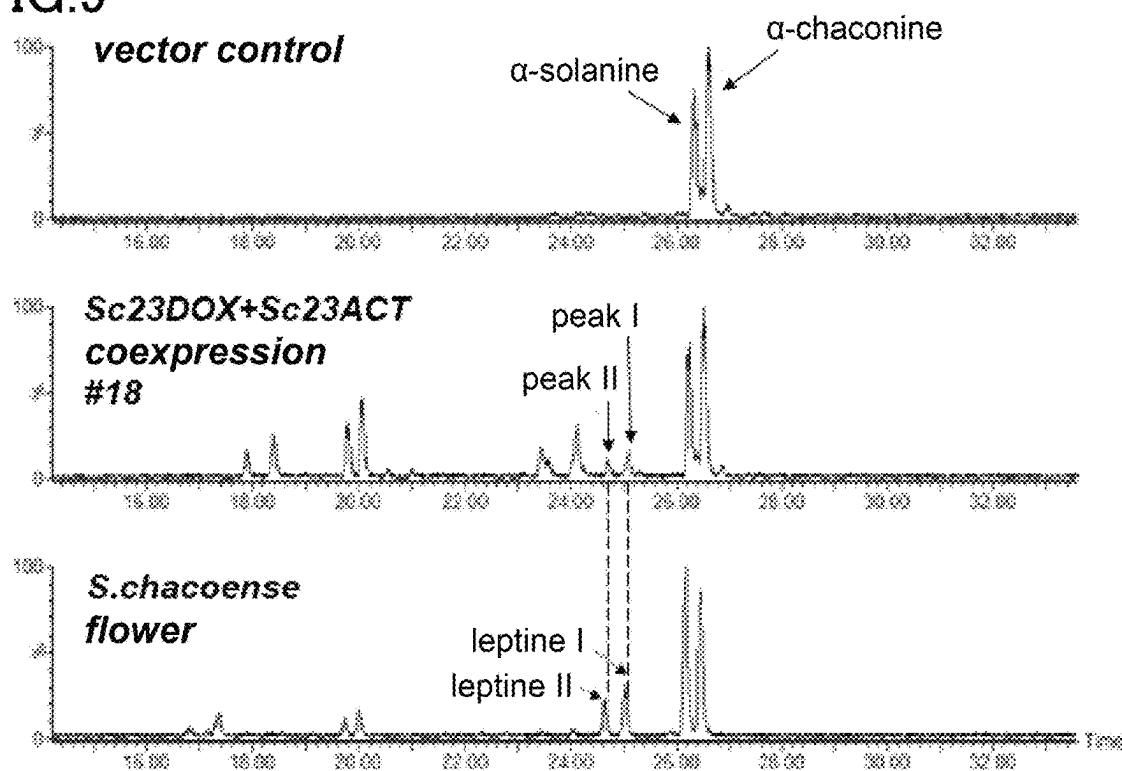


FIG.10

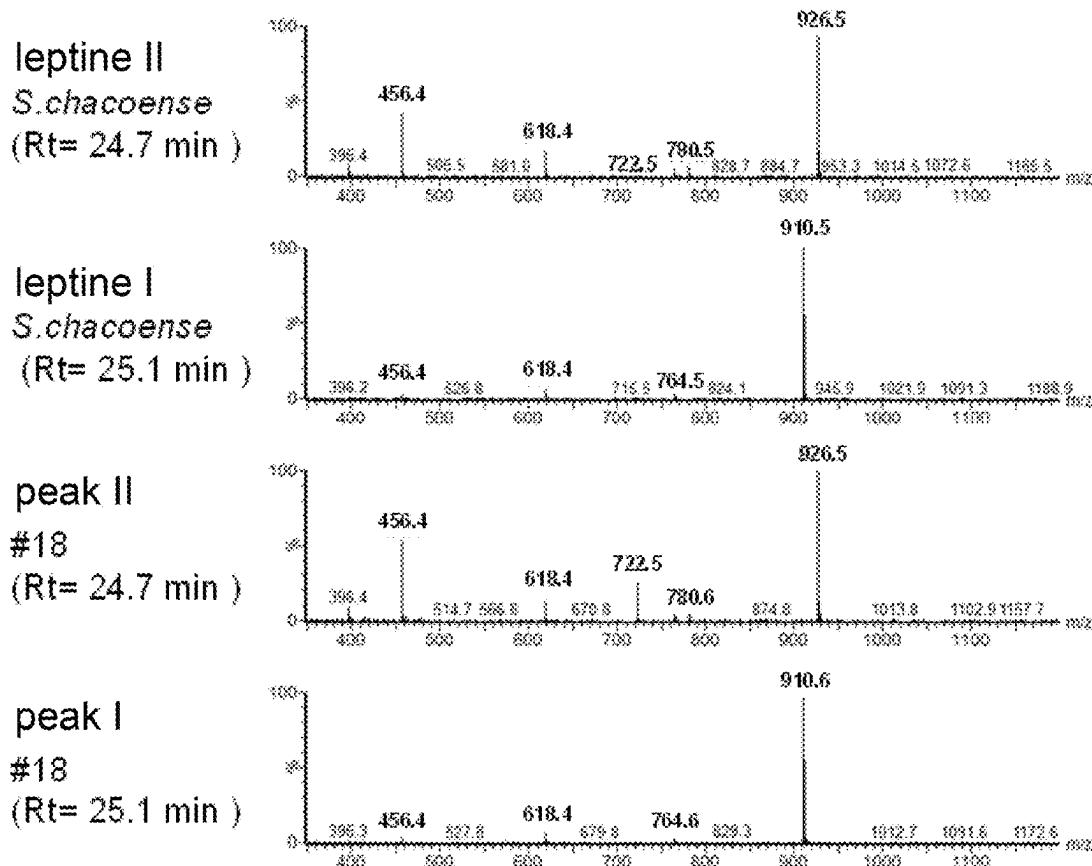


FIG.11

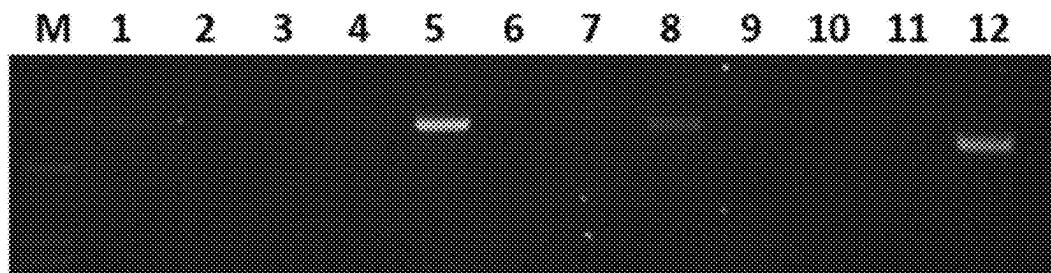


FIG.12

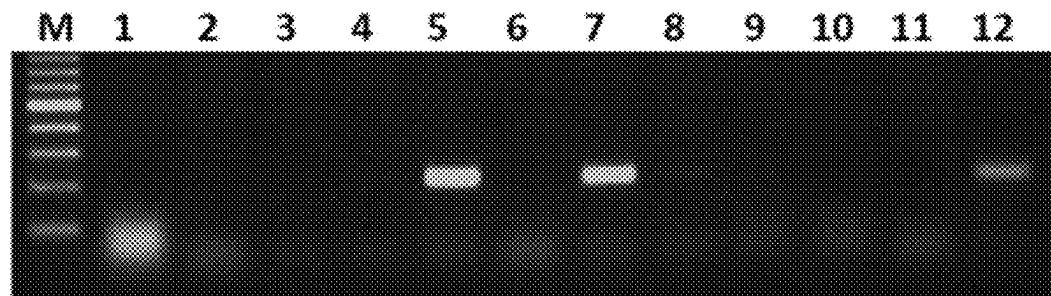


FIG.13

97H32-6

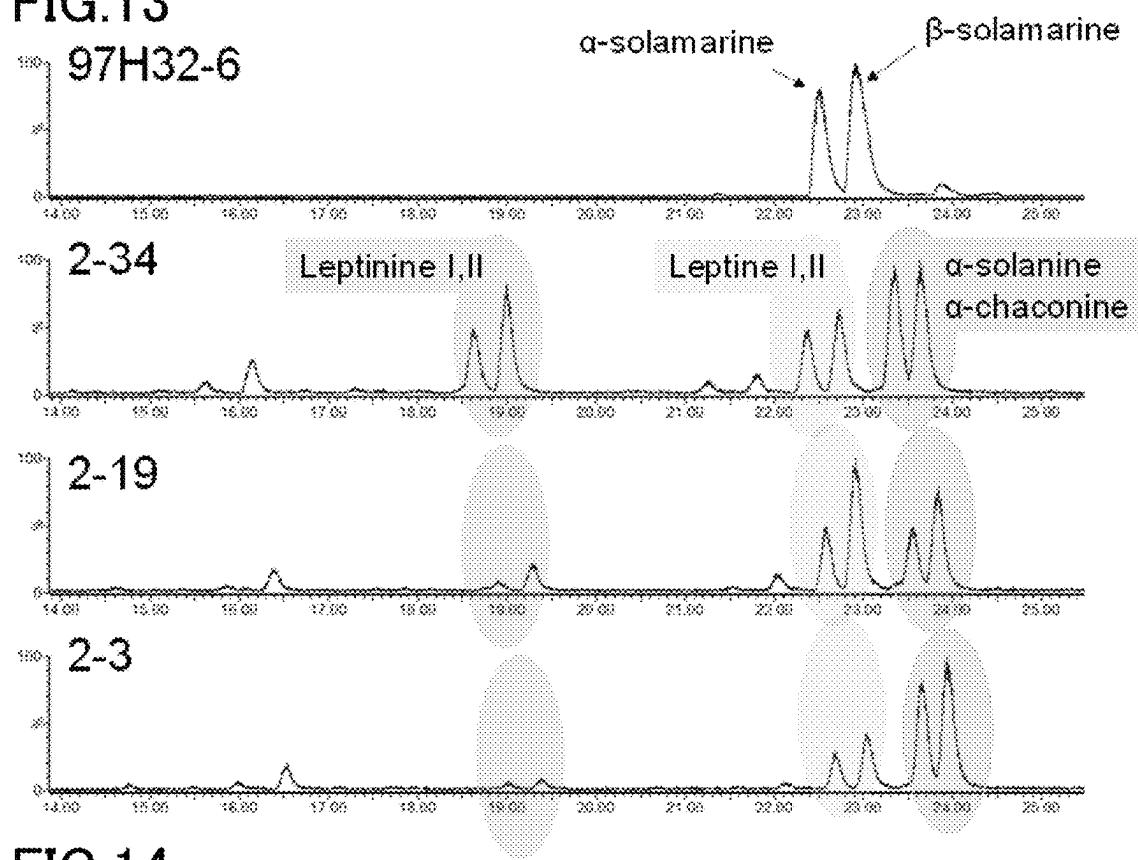
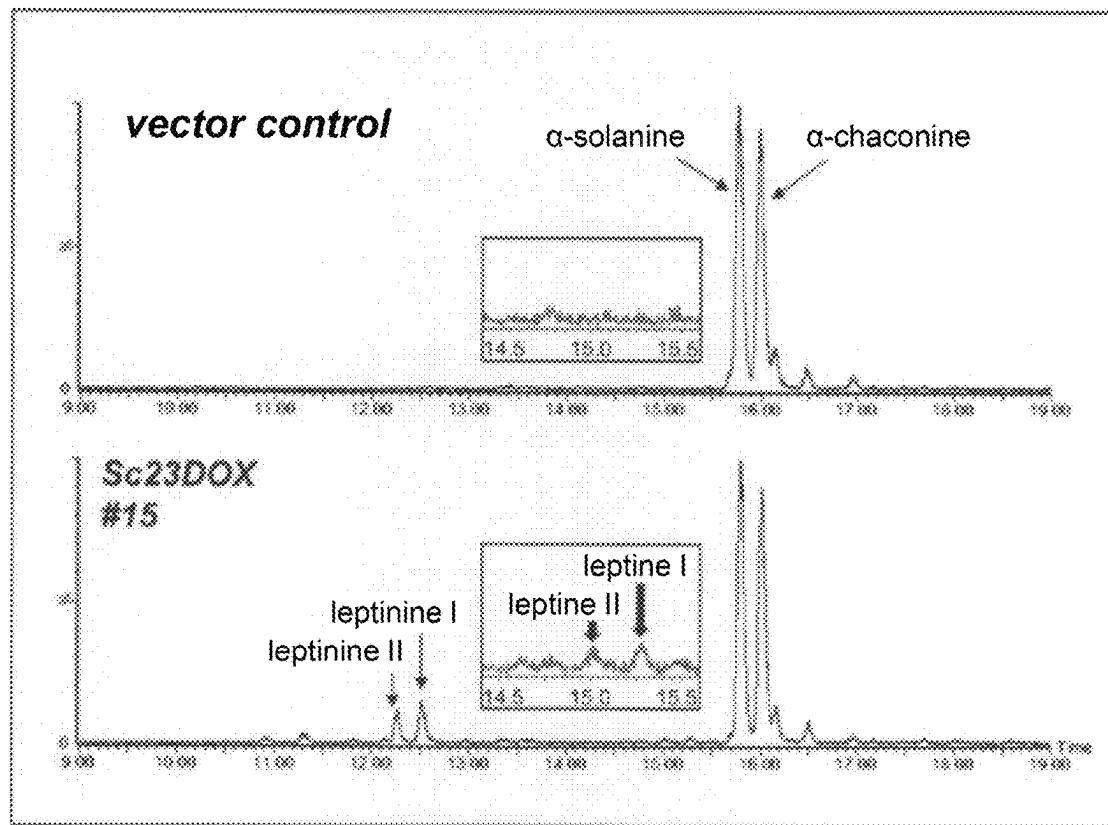
**FIG.14**

FIG.15

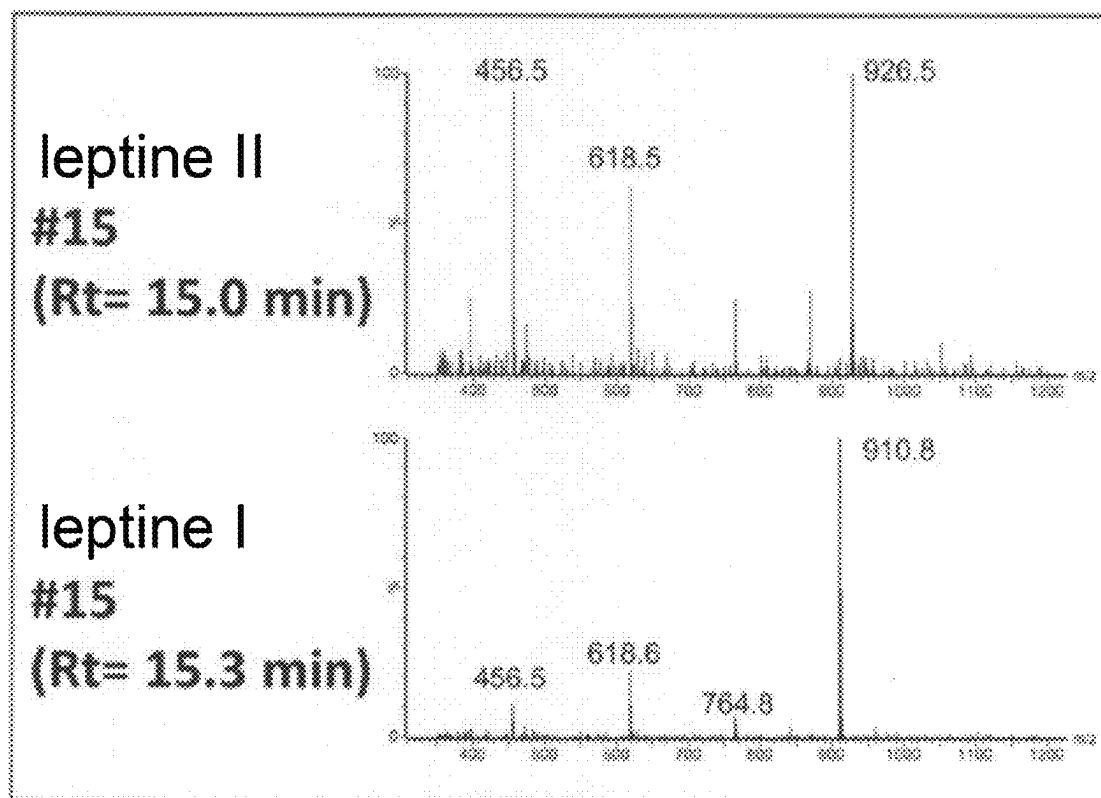


FIG. 16A

FIG.16B

ScACTPromoter : SEQ ID NO. 37	661	[Sequence Data]	660
M6ACTPromoter : SEQ ID NO. 34	661	[Sequence Data]	660
KonafubukiACTPromoterA : SEQ ID NO. 38	661	[Sequence Data]	660
KonafubukiACTPromoterB : SEQ ID NO. 39	660	[Sequence Data]	659
TuberosumACTPromotoer : SEQ ID NO. 40	660	[Sequence Data]	659
ScACTPromoter : SEQ ID NO. 37	661	[Sequence Data]	720
M6ACTPromoter : SEQ ID NO. 34	661	[Sequence Data]	720
KonafubukiACTPromoterA : SEQ ID NO. 38	661	[Sequence Data]	719
KonafubukiACTPromoterB : SEQ ID NO. 39	660	[Sequence Data]	718
TuberosumACTPromotoer : SEQ ID NO. 40	660	[Sequence Data]	718
ScACTPromoter : SEQ ID NO. 37	721	[Sequence Data]	720
M6ACTPromoter : SEQ ID NO. 34	721	[Sequence Data]	720
KonafubukiACTPromoterA : SEQ ID NO. 38	721	[Sequence Data]	720
KonafubukiACTPromoterB : SEQ ID NO. 39	720	[Sequence Data]	719
TuberosumACTPromotoer : SEQ ID NO. 40	720	[Sequence Data]	719
ScACTPromoter : SEQ ID NO. 37	721	[Sequence Data]	820
M6ACTPromoter : SEQ ID NO. 34	721	[Sequence Data]	820
KonafubukiACTPromoterA : SEQ ID NO. 38	721	[Sequence Data]	820
KonafubukiACTPromoterB : SEQ ID NO. 39	720	[Sequence Data]	819
TuberosumACTPromotoer : SEQ ID NO. 40	720	[Sequence Data]	819
ScACTPromoter : SEQ ID NO. 37	841	[Sequence Data]	840
M6ACTPromoter : SEQ ID NO. 34	841	[Sequence Data]	840
KonafubukiACTPromoterA : SEQ ID NO. 38	841	[Sequence Data]	840
KonafubukiACTPromoterB : SEQ ID NO. 39	840	[Sequence Data]	839
TuberosumACTPromotoer : SEQ ID NO. 40	840	[Sequence Data]	839
ScACTPromoter : SEQ ID NO. 37	961	[Sequence Data]	960
M6ACTPromoter : SEQ ID NO. 34	961	[Sequence Data]	960
KonafubukiACTPromoterA : SEQ ID NO. 38	961	[Sequence Data]	960
KonafubukiACTPromoterB : SEQ ID NO. 39	960	[Sequence Data]	959
TuberosumACTPromotoer : SEQ ID NO. 40	960	[Sequence Data]	959
ScACTPromoter : SEQ ID NO. 37	961	[Sequence Data]	1020
M6ACTPromoter : SEQ ID NO. 34	961	[Sequence Data]	1020
KonafubukiACTPromoterA : SEQ ID NO. 38	961	[Sequence Data]	1020
KonafubukiACTPromoterB : SEQ ID NO. 39	960	[Sequence Data]	1019
TuberosumACTPromotoer : SEQ ID NO. 40	960	[Sequence Data]	1019
ScACTPromoter : SEQ ID NO. 37	1021	[Sequence Data]	1079
M6ACTPromoter : SEQ ID NO. 34	1021	[Sequence Data]	1079
KonafubukiACTPromoterA : SEQ ID NO. 38	1021	[Sequence Data]	1079
KonafubukiACTPromoterB : SEQ ID NO. 39	1020	[Sequence Data]	1078
TuberosumACTPromotoer : SEQ ID NO. 40	1020	[Sequence Data]	1078
ScACTPromoter : SEQ ID NO. 37	1050	[Sequence Data]	1139
M6ACTPromoter : SEQ ID NO. 34	1050	[Sequence Data]	1139
KonafubukiACTPromoterA : SEQ ID NO. 38	1050	[Sequence Data]	1139
KonafubukiACTPromoterB : SEQ ID NO. 39	1079	[Sequence Data]	1138
TuberosumACTPromotoer : SEQ ID NO. 40	1079	[Sequence Data]	1138
ScACTPromoter : SEQ ID NO. 37	1140	[Sequence Data]	1199
M6ACTPromoter : SEQ ID NO. 34	1140	[Sequence Data]	1199
KonafubukiACTPromoterA : SEQ ID NO. 38	1140	[Sequence Data]	1199
KonafubukiACTPromoterB : SEQ ID NO. 39	1138	[Sequence Data]	1198
TuberosumACTPromotoer : SEQ ID NO. 40	1138	[Sequence Data]	1198

FIG. 16C

FIG.17A

Sc PromoterA : SEQ ID NO. 44	1 GCGTCTGCAATTTTGTTTTTAACGAAATTGGTGTGATTGAGGGATTTTGTGTCAGATGCG 60
Sc PromoterB : SEQ ID NO. 45	1 CGCTCGCAGTTTCTGTTTTTACGAAATTGGTGTGATTGAGGGATTTTGTGTCAGATGCG 60
M6 TPromoter : SEQ ID NO. 41	1 GCGTCTGCAATTTTGTTTTTAACGAAATTGGTGTGATTGAGGGATTTTGTGTCAGATGCG 60
Sassy PromoterA : SEQ ID NO. 47	0 ----- 0
Konafubuki PromoterA : SEQ ID NO. 49	0 ----- 0
Sassy PromoterB : SEQ ID NO. 48	0 ----- 0
Sc PromoterA : SEQ ID NO. 44	61 AATATTTCTGTAATAATGGCTTATTAATGGTGTGATTGAGGGATTTTGTGTCAGATGCG 120
Sc PromoterB : SEQ ID NO. 45	61 AATATTTCTGTAATAATGGCTTATTAATGGTGTGATTGAGGGATTTTGTGTCAGATGCG 120
M6 TPromoter : SEQ ID NO. 41	61 AATATTTCTGTAATAATGGCTTATTAATGGTGTGATTGAGGGATTTTGTGTCAGATGCG 120
Sassy PromoterA : SEQ ID NO. 47	0 ----- 0
Konafubuki PromoterA : SEQ ID NO. 49	0 ----- 0
Sassy PromoterB : SEQ ID NO. 48	0 ----- 0
Sc PromoterA : SEQ ID NO. 44	122 CGTGGCGAACTTGTAGGTGTTTTTGAAAGTTGGGGACCGCTTGTGATTTGCTTAAAG 180
Sc PromoterB : SEQ ID NO. 45	122 CGTGGCGAACTTGTAGGTGTTTTTGAAAGTTGGGGACCGCTTGTGATTTGCTTAAAG 180
M6 TPromoter : SEQ ID NO. 41	122 CGTGGCGAACTTGTAGGTGTTTTTGAAAGTTGGGGACCGCTTGTGATTTGCTTAAAG 180
Sassy PromoterA : SEQ ID NO. 47	0 ----- 0
Konafubuki PromoterA : SEQ ID NO. 49	0 ----- 0
Sassy PromoterB : SEQ ID NO. 48	0 ----- 0
Sc PromoterA : SEQ ID NO. 44	183 TTTTTTTCGAAATACTTAAATTTCTGTAATACTTGTGAGGTGTGAAATTGCTTAAAG 240
Sc PromoterB : SEQ ID NO. 45	183 -TTTTTTCGAAATACTTAAATTTCTGTAATACTTGTGAGGTGTGAAATTGCTTAAAG 240
M6 TPromoter : SEQ ID NO. 41	183 -TTTTTTCGAAATACTTAAATTTCTGTAATACTTGTGAGGTGTGAAATTGCTTAAAG 240
Sassy PromoterA : SEQ ID NO. 47	0 ----- 0
Konafubuki PromoterA : SEQ ID NO. 49	0 ----- 0
Sassy PromoterB : SEQ ID NO. 48	0 ----- 0
Sc PromoterA : SEQ ID NO. 44	244 TTGAAACGGCTGTGTTAAATGCTTGTGAAATTCGCGTTTGTGTTTTTGTGTCAGC 300
Sc PromoterB : SEQ ID NO. 45	244 TTGAAACGGCTGTGTTAAATGCTTGTGAAATTCGCGTTTGTGTTTTTGTGTCAGC 300
M6 TPromoter : SEQ ID NO. 41	244 TTGAAACGGCTGTGTTAAATGCTTGTGAAATTCGCGTTTGTGTTTTTGTGTCAGC 300
Sassy PromoterA : SEQ ID NO. 47	0 ----- 0
Konafubuki PromoterA : SEQ ID NO. 49	0 ----- 0
Sassy PromoterB : SEQ ID NO. 48	0 ----- 0
Sc PromoterA : SEQ ID NO. 44	305 TCAAAATTGTTTGTGAAATAATGTTGATTTGTAAATTTGCTTAAACGCTTTT 260
Sc PromoterB : SEQ ID NO. 45	305 TCAAAATTGTTTGTGAAATAATGTTGATTTGTAAATTTGCTTAAACGCTTTT 260
M6 TPromoter : SEQ ID NO. 41	305 TCAAAATTGTTTGTGAAATAATGTTGATTTGTAAATTTGCTTAAACGCTTTT 260
Sassy PromoterA : SEQ ID NO. 47	0 ----- 0
Konafubuki PromoterA : SEQ ID NO. 49	0 ----- 0
Sassy PromoterB : SEQ ID NO. 48	0 ----- 0
Sc PromoterA : SEQ ID NO. 44	366 CGCTCTTATTTTTTAAATGCTGTAAAGCTTATTCGAACTTGTGAACTTGTGAAAT 420
Sc PromoterB : SEQ ID NO. 45	366 CGCTCTTATTTTTTAAATGCTGTAAAGCTTATTCGAACTTGTGAACTTGTGAAAT 420
M6 TPromoter : SEQ ID NO. 41	366 CGCTCTTATTTTTTAAATGCTGTAAAGCTTATTCGAACTTGTGAACTTGTGAAAT 420
Sassy PromoterA : SEQ ID NO. 47	0 ----- 0
Konafubuki PromoterA : SEQ ID NO. 49	0 ----- 0
Sassy PromoterB : SEQ ID NO. 48	0 ----- 0
Sc PromoterA : SEQ ID NO. 44	427 ATATATACTGAAATTTGCGCTGTAAAGCTTATTCGAACTTGTGAACTTGTGAAAT 480
Sc PromoterB : SEQ ID NO. 45	427 ATATATACTGAAATTTGCGCTGTAAAGCTTATTCGAACTTGTGAACTTGTGAAAT 480
M6 TPromoter : SEQ ID NO. 41	427 ATATATACTGAAATTTGCGCTGTAAAGCTTATTCGAACTTGTGAACTTGTGAAAT 480
Sassy PromoterA : SEQ ID NO. 47	0 ----- 0
Konafubuki PromoterA : SEQ ID NO. 49	0 ----- 0
Sassy PromoterB : SEQ ID NO. 48	0 ----- 0

FIG. 17B

FIG. 17C

FIG.17D

Sc PromoterA : SEQ ID NO. 44	1452	TGATATATGGACCGCTATTTCTTCATTTTTAAAGTAAATATCCTGT-----	1452
Sc PromoterB : SEQ ID NO. 45	1453	TCATATATGGACCGCTATTTCTTCATTTTTAAAGTAAATATCCTGT-----	1453
M6 TPromoter : SEQ ID NO. 41	1204	--AATATATGGACCGCTATTTCTTCATTTTTAAAGTAAATATCCTGT-----	1204
Sassy PromoterA : SEQ ID NO. 47	268	-----GTTAGGGGTTTG 268	
Konafubuki PromoterA : SEQ ID NO. 49	268	-----GTTAGGGGTTTG 268	
Sassy PromoterB : SEQ ID NO. 48	268	-----GTTAGGGGTTTG 268	
Sc PromoterA : SEQ ID NO. 44	1452	-----	1452
Sc PromoterB : SEQ ID NO. 45	1453	-----	1453
M6 TPromoter : SEQ ID NO. 41	1204	-----	1204
Sassy PromoterA : SEQ ID NO. 47	429	CCCCCGGTTTTTAAAGGATTTTAAAGGTTTTTAAAGGAAAGGAAAGGAAAGA 429	
Konafubuki PromoterA : SEQ ID NO. 49	429	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGGAAAGA 429	
Sassy PromoterB : SEQ ID NO. 48	429	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 429	
Sc PromoterA : SEQ ID NO. 44	1452	-----	1452
Sc PromoterB : SEQ ID NO. 45	1453	-----	1453
M6 TPromoter : SEQ ID NO. 41	1204	-----	1204
Sassy PromoterA : SEQ ID NO. 47	429	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 429	
Konafubuki PromoterA : SEQ ID NO. 49	429	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 429	
Sassy PromoterB : SEQ ID NO. 48	429	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 429	
Sc PromoterA : SEQ ID NO. 44	1452	-----	1452
Sc PromoterB : SEQ ID NO. 45	1453	-----	1453
M6 TPromoter : SEQ ID NO. 41	1204	-----	1204
Sassy PromoterA : SEQ ID NO. 47	549	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 549	
Konafubuki PromoterA : SEQ ID NO. 49	549	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 549	
Sassy PromoterB : SEQ ID NO. 48	549	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 549	
Sc PromoterA : SEQ ID NO. 44	1452	-----	1452
Sc PromoterB : SEQ ID NO. 45	1453	-----	1453
M6 TPromoter : SEQ ID NO. 41	1204	-----	1204
Sassy PromoterA : SEQ ID NO. 47	669	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 669	
Konafubuki PromoterA : SEQ ID NO. 49	669	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 669	
Sassy PromoterB : SEQ ID NO. 48	669	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 669	
Sc PromoterA : SEQ ID NO. 44	1452	-----	1452
Sc PromoterB : SEQ ID NO. 45	1453	-----	1453
M6 TPromoter : SEQ ID NO. 41	1204	-----	1204
Sassy PromoterA : SEQ ID NO. 47	669	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 669	
Konafubuki PromoterA : SEQ ID NO. 49	669	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 669	
Sassy PromoterB : SEQ ID NO. 48	669	CCCCGGTTTTTAAAGGATTTTAAAGGAAAGGAAAGGAAAGGAAAGA 669	
Sc PromoterA : SEQ ID NO. 44	1452	-----	1452
Sc PromoterB : SEQ ID NO. 45	1453	-----	1453
M6 TPromoter : SEQ ID NO. 41	1204	-----	1204
Sassy PromoterA : SEQ ID NO. 47	729	AATAGCTGGTTTCAATGGTAGCTGTTGCACGCCATGTTCTTATCTACT 729	
Konafubuki PromoterA : SEQ ID NO. 49	729	AATAGCTGGTTTCAATGGTAGCTGTTGCACGCCATGTTCTTATCTACT 729	
Sassy PromoterB : SEQ ID NO. 48	729	AATAGCTGGTTTCAATGGTAGCTGTTGCACGCCATGTTCTTATCTACT 729	
Sc PromoterA : SEQ ID NO. 44	1452	-----	1452
Sc PromoterB : SEQ ID NO. 45	1453	-----	1453
M6 TPromoter : SEQ ID NO. 41	1204	-----	1204
Sassy PromoterA : SEQ ID NO. 47	769	AATGGTTTTATTATGTTGCTGTTGCACGCCATGTTCTTATCTACT 769	
Konafubuki PromoterA : SEQ ID NO. 49	769	AATGGTTTTATTATGTTGCTGTTGCACGCCATGTTCTTATCTACT 769	
Sassy PromoterB : SEQ ID NO. 48	769	AATGGTTTTATTATGTTGCTGTTGCACGCCATGTTCTTATCTACT 769	

FIG.17E

Sc PromoterA : SEQ ID NO. 44	1453	-----	TTTTCGAAATCGTGAATACTTTCGAAAC	1483
Sc PromoterB : SEQ ID NO. 45	1454	-----	TTTTCGAAATCGTGAATACTTTCGAAAC	1484
M6 TPromoter : SEQ ID NO. 41	1305	-----	TTTTCGAAATCGTGAATACTTTCGAAAC	1335
Sassy PromoterA : SEQ ID NO. 47	790	GAGGGCTATTTCGTTCTGCAACGTTTGTGAAATCTGATTAATTTT	847	
Konafubuki PromoterA : SEQ ID NO. 49	790	CGAGGCTATTTCGTTCTGCAACGTTTGTGAAATCTGATTAATTTT	847	
Sassy PromoterB : SEQ ID NO. 48	790	GAGGGCTATTTCGTTCTGCAACGTTTGTGAAATCTGATTAATTTT	847	
Sc PromoterA : SEQ ID NO. 44	1484	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	1483
Sc PromoterB : SEQ ID NO. 45	1485	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	1484
M6 TPromoter : SEQ ID NO. 41	1336	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	1335
Sassy PromoterA : SEQ ID NO. 47	848	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	908
Konafubuki PromoterA : SEQ ID NO. 49	848	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	906
Sassy PromoterB : SEQ ID NO. 48	848	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	906
Sc PromoterA : SEQ ID NO. 44	1543	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	1592
Sc PromoterB : SEQ ID NO. 45	1544	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	1593
M6 TPromoter : SEQ ID NO. 41	1336	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	1446
Sassy PromoterA : SEQ ID NO. 47	806	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	856
Konafubuki PromoterA : SEQ ID NO. 49	806	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	855
Sassy PromoterB : SEQ ID NO. 48	806	TTTTCGAAATCGTGAATACTTTCGAAAC	TTTTCGAAATCGTGAATACTTTCGAAAC	856

**PLANT HAVING ENHANCED RESISTANCE
AGAINST COLORADO POTATO BEETLE
AND METHOD FOR PRODUCING SAME,
AND METHOD FOR EVALUATING
RESISTANCE AGAINST COLORADO
POTATO BEETLE IN PLANT**

INCORPORATION BY REFERENCE

The 57,264-byte text file titled “22KC-001SequenceListing4.txt” (Creation Date: Sep. 20, 2024) is hereby incorporated by reference.

TECHNICAL FIELD

The present invention relates to a plant having increased resistance against Colorado potato beetle and to a plant cell that is capable of regenerating the plant. Also, the present invention relates to a method for producing the plant and to a composition used for increasing resistance of a plant against Colorado potato beetle. Further, the present invention relates to a method for determining resistance against Colorado potato beetle in a plant. Also, the present invention relates to a composition used for introducing a hydroxy group or an acetoxy group to position 23 of a spirostanol skeleton, or to a composition used for increasing an amount of leptinines or leptines accumulated in a plant.

BACKGROUND ART

The Colorado potato beetle (scientific name; *Leptinotarsa decemlineata*, common name: Colorado potato beetle) is an insect of the Family Chrysomelidae of the Suborder Polyphaga (*Polyphaga*) of the Order Coleoptera. The Colorado potato beetle is a pest that parasitizes plants of the Family Solanaceae and eats their leaves. It is also reported that Colorado potato beetle causes the most serious insect damage especially in potatoes (e.g., *Solanum tuberosum*) and that without any agricultural chemical that is an insecticide, the loss of 40 to 80% of the amount of production can occur (NPL 1). Colorado potato beetle (CPB) has not come to Japan, but is spreading its habitat to various areas in the world, including from North America to Europe, and Asia (NPL 1). In recent years, CPB inhabits also in China, and in 2010, it is distributed in an area of 270,000 km². In an area having a large habitat, CPB is spreading at 45 km/year (NPL 2). Therefore, breeding CPB-resistant potatoes and other products is an urgent need also in Japan and also a globally important issue.

In this regard, as a potato having resistance against CPB, *Solanum chacoense* (wild species) is known. Also, it is revealed that CPB-resistant potatoes derived from *S. chacoense* can exhibit this resistance by accumulating leptinines (NPLs 3 and 4). Further, it is expected that these leptinines are biosynthesized, via a 2-step reaction, from solanine and chaconine accumulated in usual cultivated species, potatoes (e.g., *S. tuberosum*) (NPL 5). It is suggested that genes responsible for the biosynthesis of these leptinines are located in Chromosomes 2 and 8 derived from *S. chacoense* (NPL 6).

However, this *S. chacoense* is a wild species that has not been domesticated. The yield and cultivation performance thereof are much lower than those of usual edible potatoes. This is why *S. chacoense* is not suitable for agricultural production, which is problematic. Therefore, an attempt has been made to interbreed a line derived from *S. chacoense* with usual potatoes (e.g., *S. tuberosum*) to obtain a progeny

line having CPB resistance. In order to breed a potato as an excellent commercial variety, it is necessary to remove a *S. chacoense*-derived genome region that is unnecessary for CPB resistance. Removal of that region is usually performed by back-crossing with, for example, *S. tuberosum*. As described above, however, genes responsible for leptine biosynthesis have not been identified, and also the loci information of the genes has been only roughly revealed; i.e., Chromosomes 2 and 8. Therefore, selection of individuals cannot be performed using as an indicator, for example, the presence of those genes, and a commercial variety having CPB resistance has not been obtained yet.

Meanwhile, as a method for imparting CPB resistance against plants such as potatoes or the like, conceivable is a method of introducing genes responsible for leptine biosynthesis into, for example, potatoes through gene recombination, genome editing, or other techniques. As described above, however, the above genes have not been identified, and CPB-resistant plants have not been produced yet also by such a method.

This time, we have identified, from tomatoes, S123DOX gene encoding an enzyme that hydroxylates position 23 of a spirostanol compound. Based on this information, we have identified Sc23DOX from *Solanum chacoense* that accumulates leptines. Further, surprisingly, we have identified and discovered St23DOX that is not usually expressed, also in *Solanum tuberosum*. We have revealed that by introducing them into potatoes in the form that they can be expressed, leptinines, precursors of leptines which are CPB-resistant factors, can be produced.

In recent years, the same gene as the S123DOX gene was reported as GAME31 (PTL 1 and NPL 7). According to these reports, GAME31 is identified from tomatoes, eggplants, and *Solanum chacoense*, but as described in PTL 1, the presence of GAME31 in *Solanum tuberosum* was not able to be found.

Regarding enzymatic activities, the reports demonstrate that the enzyme hydroxylates position 23 of a spirostanol compound (PTL 1 and NPL 7) but cannot hydroxylate solanidine (NPL 7). That is, although GAME31 of potatoes was expected to hydroxylate chaconine and solanine, precursors of leptinines I and II (PTL 1), GAME32 different from GAME31 was reported to be necessary for the hydroxylation (NPL 7, FIG. 7b) and the above expectation was denied. In view thereof, the authors of PTL 1 and NPL 7 do not suggest that leptinines, precursors of leptines can be produced by introducing 23DOX/GAME31 into potatoes.

This time, the present inventors have identified gene 23ACT encoding an enzyme that acetylates a 23-hydroxylated spirostanol compound. These gene and enzyme reaction are new that have never been reported in related art documents, including PTL 1 and NPL 7.

CITATION LIST

Patent Literature

- PTL 1: International Publication No. WO2018146678A1
IL2018050142W GLYCOALKALOID METABOLISM
ENZYMES (GAMES) AND USES THEREOF
Non-Patent Literature
NPL 1: Maharijaya and Vosman (2015) Euphytica 4:133-142
NPL 2: Liu et al. (2012) Entomologia Experimentalis et Applicata 143:207-217
NPL 3: Sturckow and Low (1961) Entomologia Experimentalis et Applicata 4:133-142

- NPL 4: Sinden et al. (1986) Environmental Entomology 15:1057-1062
 NPL 5: Ronning et al. (1999) Theor Appl Genet. 98:39-46
 NPL 6: Sagredo et al. (2006) Theor Appl Genet. 114:131-42
 NPL 7: Cardenas et al. (2019) NATURE COMMUNICATIONS 10:5169

SUMMARY OF INVENTION

Technical Problem

The present invention has been made in view of the above problems in the art. An object of the present invention is to identify a gene responsible for imparting CPB resistance to a plant; i.e., a gene responsible for biosynthesis of leptines. Another object of the present invention is to provide a method for efficiently producing a plant having increased resistance against CPB by using a gene identified. Still another object of the present invention is to provide a method for efficiently determining resistance against CPB in a plant by using an indicator, for example, the presence of the gene.

Solution to Problem

As described above, in *S. chacoense* that is a CPB-resistant potato, leptines that impart the resistance are assumed to be biosynthesized from solanine and chaconine (compounds having a solanidine skeleton). Regarding this biosynthesis, the present inventors focused on the metabolic process of α -tomatine (a compound having a spirosolane skeleton) in a tomato (*S. lycopersicum*), which is a plant of the same Family Solanaceae (see the upper part of FIG. 1). We assumed that in *S. chacoense*, position-23 hydroxylase (hereinafter referred to also as "23DOX") and position-23 acetyltransferase (hereinafter referred to also as "23ACT") similar to those in the metabolic process of α -tomatine in the tomato would be involved with biosynthesis of leptines through production of leptinines using α -solanine and α -chaconine as substrates (see the lower part of FIG. 1).

Then, the present inventors first tried to identify genes encoding these enzymes for the respective *S. lycopersicum* and *S. chacoense*. Although it has been revealed that the 23DOX and the 23ACT were involved with the metabolic process of α -tomatine, these sequences have not been revealed. Therefore, we first selected, from the expression database of the tomato, gene sequences that would be assumed to be involved with the metabolic process, and determined the full-length ORF sequences thereof. Then, based on the sequence of *S. lycopersicum* obtained, we screened for homologous genes in *S. chacoense* and also determined the full-length ORF sequences thereof through RACE method.

Then, enzymes encoded by the respective genes of *S. lycopersicum* and *S. chacoense* that we were able to obtain in such a manner were synthesized in *Escherichia coli*, and were analyzed for their enzymatic activity in vitro. As a result, it is revealed that 23DOX of *S. lycopersicum* and 23DOX of *S. chacoense* (which may also be referred to as "S123DOX" and "Sc23DOX", respectively) were able to each introduce a hydroxy group to position 23 of α -tomatine, and 23ACT of *S. lycopersicum* and 23ACT of *S. chacoense* (which may also be referred to as "S123ACT" and "Sc23ACT", respectively) were able to acetylate the hydroxy group.

Contrary to previous expectations, however, when α -solanine and other compounds were used also as a substrate,

a hydroxy group was not introduced by the 23DOX to position 23 of the above compound, nor was the hydroxy group acetylated by the 23ACT, failing to produce leptines.

Meanwhile, production of leptinines was detected in a potato (*S. tuberosum*) to which the 23DOX gene had been introduced, and production of leptines was confirmed in the potato to which the 23DOX gene and the 23ACT gene had been introduced.

In view of the above, it is revealed that in *S. chacoense*, leptines are not biosynthesized from compounds having a solanidine skeleton (α -solanine and α -chaconine) differing from the previous expectations, but leptines are biosynthesized through the process including introducing a hydroxy group to position 23 of compounds having a spirosolane skeleton, followed by introduction of an acetoxy group, and further converting the spirosolane skeletons of these compounds to a solanidine skeleton.

Also, we produced primer sets that recognized the Sc23DOX gene and the Sc23ACT gene. We further produced crosses between *S. chacoense* and diploid potatoes that do not produce leptines. These crosses were analyzed through PCR using the primer sets and were analyzed for the amount of leptines generated. As a result, it was confirmed that the presence of the DNA markers (the 23DOX gene and the 23ACT gene of *S. chacoense*) was in agreement to the accumulation of leptines in terms of genetic behaviors.

When we further analyzed the presence or absence of the 23DOX gene and the 23ACT gene in *S. tuberosum* having no CPB resistance, surprisingly, it was revealed that the *S. tuberosum* had genes encoding proteins having high sequence identities to the 23DOX and the 23ACT (St23DOX and St23ACT).

Also, proteins encoded by these genes were synthesized in *Escherichia coli* and were analyzed for their enzymatic activities in vitro. As a result, it was revealed that, similarly with the above-described S123DOX and Sc23DOX, the St23DOX was able to introduce a hydroxy group to position 23 of α -tomatine. Further, it was also revealed that similar to the above-described S123ACT and Sc23ACT, the St23ACT was able to acetylate the hydroxy group.

On the basis of the above findings, the present invention has been completed. That is, the present invention provides the followings in detail.

- <1> A composition that is used for introducing a hydroxy group to position 23 of a spirosolane skeleton, the composition including:
 at least one DNA selected from the group consisting of
 (a) to (d) below:
- (a) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6;
 - (b) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6, and has an activity to hydroxylate position 23 of a spirosolane skeleton;
 - (c) DNA encoding a protein that consists of an amino acid sequence identical to the amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6 except that one or two or more amino acids are substituted, deleted, added, and/or inserted, and has the activity to hydroxylate the position 23 of the spirosolane skeleton; and
 - (d) DNA that hybridizes under stringent conditions with DNA consisting of a complementary sequence to a nucleotide sequence as set forth in

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SEQ ID NO: 1, 3, or 5, and encodes a protein having an activity to hydroxylate position 23 of a spirostanol skeleton.

<2> A composition that is used for introducing an acetoxy group to position 23 of a spirostanol skeleton, the composition including:

at least one DNA selected from the group consisting of the (a) to (d) above and at least one DNA selected from the group consisting of (e) to (h) below:

(e) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12;

(f) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12, and has an activity to acetylate a hydroxy group of position 23 of a spirostanol skeleton;

(g) DNA encoding a protein that consists of an amino acid sequence identical to the amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12 except that one or two or more amino acids are substituted, deleted, added, and/or inserted, and has an activity to acetylate a hydroxy group of the position 23 of the spirostanol skeleton; and

(h) DNA that hybridizes under stringent conditions with DNA consisting of a complementary sequence to a nucleotide sequence as set forth in SEQ ID NO: 7, 9, or 11, and encodes a protein having an activity to acetylate a hydroxy group of position 23 of a spirostanol skeleton.

<3> A composition that is used for increasing an accumulation amount of leptinine in a plant, the composition including:

at least one DNA selected from the group consisting of the (a) to (d) above.

<4> A composition that is used for increasing an accumulation amount of lepine in a plant, the composition including:

at least one DNA selected from the group consisting of the (a) to (h) above.

<5> A composition that is used for increasing resistance of a plant against Colorado potato beetle, the composition including:

at least one DNA selected from the group consisting of the (a) to (h) above.

<6> A transformed plant cell that is capable of regenerating a plant body having increased resistance against Colorado potato beetle, the transformed plant cell including:

at least one DNA selected from the group consisting of the (a) to (h) above, the at least one DNA being introduced to the transformed plant cell.

<7> A transformed plant having increased resistance against Colorado potato beetle, wherein the transformed plant is regenerated from the transformed plant cell according to <6>.

<8> A method for producing a plant having increased resistance against Colorado potato beetle, the method including:

introducing at least one DNA selected from the group consisting of the (a) to (h) above, to a plant cell; and regenerating a plant from a transformed plant cell to which the DNA is introduced in the introducing.

<9> A method for determining resistance against Colorado potato beetle in a plant, the method including:

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detecting, in a plant to be tested, presence or expression of at least one DNA selected from the group consisting of the (a) to (d) above and at least one DNA selected from the group consisting of the (e) to (h) above; and

when the presence or expression of the DNAs is detected in the detecting, determining that the plant to be tested has resistance against Colorado potato beetle.

<10> A method for producing a plant having resistance against Colorado potato beetle, the method including: crossing a plant having resistance against Colorado potato beetle, with an arbitrary plant;

determining the resistance against Colorado potato beetle in a plant obtained in the crossing, by the method according to <9>, and selecting a plant that is determined to have the resistance against Colorado potato beetle.

<11> A method for producing a plant having resistance against Colorado potato beetle, the method including: crossing a plant having at least one DNA selected from the group consisting of the (a) to (d) above, with a plant having at least one DNA selected from the group consisting of the (e) to (h) above;

determining the resistance against Colorado potato beetle in a plant obtained in the crossing, by the method according to <9>, and selecting a plant that is determined to have the resistance against Colorado potato beetle.

The nucleotide sequence of the 23DOX gene derived from *S. chacoense* and the amino acid sequence of the protein encoded by the aforementioned sequence are shown in SEQ ID NO: 1 and SEQ ID NO: 2, respectively. The nucleotide sequence of the 23DOX gene derived from *S. tuberosum* and the amino acid sequence of the protein encoded by the aforementioned sequence are shown in SEQ ID NO: 3 and SEQ ID NO: 4, respectively. The nucleotide sequence of the 23DOX gene derived from *S. lycopersicum* and the amino acid sequence of the protein encoded by the aforementioned sequence are shown in SEQ ID NO: 5 and SEQ ID NO: 6, respectively.

The nucleotide sequence of the 23ACT gene derived from *S. chacoense* and the amino acid sequence of the protein encoded by the aforementioned sequence are shown in SEQ ID NO: 7 and SEQ ID NO: 8, respectively. The nucleotide sequence of the 23ACT gene derived from *S. tuberosum* and the amino acid sequence of the protein encoded by the aforementioned sequence are shown in SEQ ID NO: 9 and SEQ ID NO: 10, respectively. The nucleotide sequence of the 23ACT gene derived from *S. lycopersicum* and the amino acid sequence of the protein encoded by the aforementioned sequence are shown in SEQ ID NO: 11 and SEQ ID NO: 12, respectively.

Advantageous Effects of Invention

Use of the hydroxylase gene and/or acetyltransferase gene identified in the present invention can introduce a hydroxy group or an acetoxy group to position 23 of a spirostanol skeleton, and can also produce leptinine or lepine. According to the present invention, enhancement of biosynthesis of lepine and accumulation of lepine can increase, for example, resistance against CPB in a plant. That is, a plant having increased resistance against CPB can be efficiently provided. According to the present invention, for example,

resistance against CPB in a plant can also be efficiently determined by using expression or presence of the gene as an indicator.

BRIEF DESCRIPTION OF DRAWINGS

FIG. 1 is a schematic view showing glycoalkaloids in tomato (*S. lycopersicum*), CPB-resistant potato (*S. chacoense*), and CPB-nonresistant potato (*S. tuberosum*). In the enclosing line in the figure, steroid glycoalkaloid produced in each solanaceous plant is shown, and “R” at position 3 of a spirostanolone skeleton and position 3 of a solanidine skeleton in the left side of the figure means saccharide complexes that bind to these skeletons via an oxygen atom. The upper part in the figure (in the enclosing line of *S. lycopersicum*) shows that, in the metabolic process of α -tomatine (a compound having a spirostanolone skeleton) in tomato, a hydroxy group is introduced to 23 position of the skeleton by 23-position hydroxylase (23DOX), and an acetyl group is further introduced to the hydroxy group by 23-position acetyltransferase (23ACT);

FIG. 2 is chart data of LC-MS showing the results of the 23-position hydroxylase activity detected using crude extraction fractions of *E. coli* (in the figure, the respective signs are shown as “S123DOX”, “Sc23DOX”, and “St23DOX”) to which a vector for expressing 23DOX derived from *S. lycopersicum*, a vector for expressing 23DOX derived from *S. chacoense*, and a vector for expressing 23DOX derived from *S. tuberosum* are each introduced. In the figure, the longitudinal axis shows intensity and the horizontal axis shows retention time (regarding the signs in the figure, the same is applied to FIGS. 4, 7, 9, 13, and 14);

FIG. 3 is the mass spectra of the peaks (1) to (4) shown in FIG. 2. In the figure, the longitudinal axis shows intensity and the horizontal axis shows the mass-to-charge ratio (regarding the signs in the figure, the same is applied to FIGS. 5, 8, 10, and 15);

FIG. 4 is chart data of LC-MS showing the results of the 23-position acetyltransferase detected using crude extraction fractions of *E. coli* (in the figure, the respective signs are shown as “S123ACT”, “Sc23ACT”, “St23ACT”, and “23 hydroxy-tomatine+negative control”) to which a vector for expressing 23ACT derived from *S. lycopersicum*, a vector for expressing 23ACT derived from *S. chacoense*, a vector for expressing 23ACT derived from *S. tuberosum*, and an empty vector are each introduced. In the figure, “23 acetoxy-tomatine” shows chart data of its specimen;

FIG. 5 is the mass spectra of the peaks (1) to (4) shown in FIG. 4;

FIG. 6 is a view illustrating the structure of a vector used for transformation. The figure shows a right border (RB), a left border (LB), the inner structures in the borders, and a restriction enzyme recognition sequence site in T-DNA of a gene site to be introduced;

FIG. 7 is chart data of LC-MS showing the results of analyzing CPB-nonresistant potato (in the figure, “*S. tuberosum*”), potato that has expressed Sc23DOX (in the figure, “*S. tuberosum* Sc23DOX-ox-#9”), and the flower of CPB-resistant potato (in the figure, “the flower of *S. chacoense*”);

FIG. 8 is the mass spectra of the peaks (1) to (4) shown in FIG. 7;

FIG. 9 is chart data of LC-MS showing the results of analyzing CPB-nonresistant potato (in the figure, “vector control”) to which an empty vector is introduced, potato that has expressed Sc23DOX and Sc23ACT (in the figure,

“Sc23DOX+Sc23ACT coexpression”), and the flower of CPB-resistant potato (in the figure, “the flower of “*S. chacoense*”);

FIG. 10 is mass spectra of the peaks (peaks I and II, and leptines I and II) shown in FIG. 9;

FIG. 11 is a photograph of gel electrophoresis, showing the results of the Sc23DOX gene detected through PCR by using, as a template, genome DNAs extracted from potato varieties exhibiting no resistance against CPB, lines used for potato breeding, and the wild species “*S. chacoense* PI 458310” that exhibits resistance against CPB. In the figure, “M” shows a 100 bp marker, “1” shows the analysis result of Irish Cobbler potato, “2” shows the result of May queen, “3” shows the analysis result of Sayaka, “4” shows the analysis result of Sassy, “5” shows the analysis result of Konafubuki, “6” shows the analysis result of Desiree, “7” shows the analysis result of 97H32-6, “8” shows the analysis result of Saikai 35, “9” shows the analysis result of Hokkai 87, “10” shows the analysis result of VTn 62-33-3, “11” shows the analysis result of W553-4, and “12” shows the analysis result of *S. chacoense* (PI 458310) (regarding the signs in the figure, the same is applied to FIG. 12);

FIG. 12 shows the results of the Sc23ACT gene detected through PCR by using, as a template, genome DNAs extracted from potato varieties exhibiting no resistance against CPB, lines used for potato breeding, and the wild species “*S. chacoense* PI 458310” that exhibits resistance against CPB;

FIG. 13 is chart data of LC-MS showing the results of analyzing steroid glycoalkaloids in hybrids, which are obtained by crossing seedlings three lines obtained from *S. chacoense* PI 458310 that has expressed leptine, with the potato line 97H32-6 that has expressed no leptine. In the figure, “97H32-6” shows the result of analyzing 97H32-6, and “2-34”, “2-19”, and “2-3” show the results of analyzing hybrids PI 458310-2×97H32-6 34, PI 458310-2×97H32-6 19, and PI 458310-2×97H32-6 3 shown in Table 1;

FIG. 14 is chart data of LC-MS showing the results of analyzing a CPB-nonresistant potato variety Konafubuki (in the figure, “vector control”) to which an empty vector is introduced and Konafubuki (in the figure, “Sc23DOX #15”) that has expressed Sc23DOX. In the enclosing line in the figure, the chart data at the retention time of from 14.5 minutes to 15.5 minutes are enlarged;

FIG. 15 is mass spectra of the peaks (leptines I and II) shown in FIG. 14;

FIG. 16A shows comparison of the sequences of about 1.7 kb promoter regions of the 23ACT genes of *S. chacoense* PI 458310, *S. chacoense* M6, Konafubuki, and Sassy, with each of the sequences stretched just before the start codon being described (part 1);

FIG. 16B shows comparison of the sequences of about 1.7 kb promoter regions of the 23ACT genes of *S. chacoense* PI 458310, *S. chacoense* M6, Konafubuki, and Sassy, with each of the sequences stretched just before the start codon being described (part 2);

FIG. 16C shows comparison of the sequences of about 1.7 kb promoter regions of the 23ACT genes of *S. chacoense* PI 458310, *S. chacoense* M6, Konafubuki, and Sassy, with each of the sequences stretched just before the start codon being described (part 3);

FIG. 17A shows comparison of the sequences of about 1 to 1.5 kb promoter regions of the 23DOX genes of *S. chacoense* PI 458310, *S. chacoense* M6, Konafubuki, and Sassy, with each of the sequences stretched just before the start codon being described (part 1);

FIG. 17B shows comparison of the sequences of about 1 to 1.5 kb promoter regions of the 23DOX genes of *S. chacoense* PI 458310, *S. chacoense* M6, Konafubuki, and Sassy, with each of the sequences stretched just before the start codon being described (part 2);

FIG. 17C shows comparison of the sequences of about 1 to 1.5 kb promoter regions of the 23DOX genes of *S. chacoense* PI 458310, *S. chacoense* M6, Konafubuki, and Sassy, with each of the sequences stretched just before the start codon being described (part 3);

FIG. 17D shows comparison of the sequences of about 1 to 1.5 kb promoter regions of the 23DOX genes of *S. chacoense* PI 458310, *S. chacoense* M6, Konafubuki, and Sassy, with each of the sequences stretched just before the start codon being described (part 4); and

FIG. 17E shows comparison of the sequences of about 1 to 1.5 kb promoter regions of the 23DOX genes of *S. chacoense* PI 458310, *S. chacoense* M6, Konafubuki, and Sassy, with each of the sequences stretched just before the start codon being described (part 5).

DESCRIPTION OF EMBODIMENTS

Regarding Composition of the Present Invention

As described in the Examples below, the present inventors isolated 23-position hydroxylase (23DOX) genes (S123DOX gene and Sc23DOX gene) in tomato (*S. lycopersicum*) and CPB-resistant potato *S. chacoense*, and identified these sequences. As a result of introducing the 23DOX gene to potato (*S. tuberosum*) that was not found to biosynthesize leptinine, it was recognized that the potato significantly biosynthesized leptinine by introducing a hydroxy group to position 23 of steroid glycoalkaloid having a spirosolane skeleton and further converting the spirosolane skeleton into a solanidine skeleton.

Moreover, the present inventors revealed that *S. tuberosum* has the 23DOX gene, but the potato was not found to biosynthesize leptinines because of its insufficient expression level.

Therefore, the present invention provides: a composition, which includes DNA encoding a protein (hereinafter is also referred to as “23DOX of the present invention”) having an activity to hydroxylate position 23 of a spirosolane skeleton and is used for introducing a hydroxy group to position 23 of the spirosolane skeleton; and a composition used for improving an accumulation amount of leptinine in a plant.

As described in Examples below, the present inventors isolated 23-position acetyltransferase (23ACT) genes (S123ACT gene and Sc23ACT gene) in *S. lycopersicum* and *S. chacoense*, and identified these sequences. As a result of introducing the 23DOX gene and the 23ACT gene to potato (*S. tuberosum*) that is not recognized to biosynthesize leptine and has no resistance against CPB, it was recognized that the potato significantly biosynthesized leptines associated with resistance against CPB by further acetylyating the hydroxy group introduced by 23DOX in the steroid glycoalkaloid having a spirosolane skeleton and then converting the spirosolane skeleton into a solanidine skeleton.

The present inventors revealed that among *S. tuberosum* varieties the varieties such as 97H32-6, Konafubuki, and the like, which are created using *S. chacoense* in the breeding process but produce no leptine, have the Sc23ACT gene.

Moreover, the present inventors revealed that *S. tuberosum* also has the 23ACT gene, but the potato was not found to biosynthesize leptines because of its insufficient expression level.

Therefore, the present invention also provides: a composition, which includes DNA encoding 23DOX of the present invention and DNA encoding a protein (hereinafter, is also referred to as “23ACT of the present invention”) having an activity to acetylate the hydroxy group of position 23 of the spirosolane skeleton, and is used for introducing an acetoxy group to position 23 of the spirosolane skeleton; a composition used for improving an accumulation amount of leptine in a plant; and a composition used for improving resistance of a plant against CPB.

In the present invention, a substrate to which a hydroxy group or an acetoxy group is introduced may be any substrate as long as it includes at least a spirosolane skeleton. Examples of the substrate include spirosolane glycosides.

In the present invention, examples of a substrate to which a hydroxy group is introduced at position 23 include α -tomatine, α -dehydrotomatine, α -solamarine, β -solamarine, and aglycones thereof, and examples of a substrate to which an acetyl group is introduced at the hydroxy group of position 23 include leptinine, 23 hydroxy tomatine, and 23 hydroxy dehydrotomatine.

In the present invention, examples of the “leptinine” include leptinine I (3β -[2-O,4-O-bis(α -L-rhamnopyranosyl)- β -D-glucopyranosyloxy]solanid-5-en-23 β -ol) and leptinine II (3β -[(3-O- β -D-glucopyranosyl-2-O- α -L-rhamnopyranosyl- β -D-galactopyranosyl)oxy]solanid-5-en-23 β -ol).

In the present invention, examples of the “leptine” include leptine I (3β -[2-O,4-O-bis(α -L-rhamnopyranosyl)- β -D-glucopyranosyloxy]solanid-5-en-23 β -ol 23-acetate) and 3β -[2-O,4-O-bis(α -L-rhamnopyranosyl)- β -D-glucopyranosyloxy]solanid-5-en-23 β -ol), and leptine II (3β -[(3-O- β -D-glucopyranosyl-2-O- α -L-rhamnopyranosyl- β -D-galactopyranosyl)oxy]solanid-5-en-23 β -acetate).

As an aspect of the “composition” of the present invention, the composition may be any composition as long as it includes, as an active component, “DNAs of the present invention” that will be described later, but may include other components. Such other components are not particularly limited. Examples thereof include sterile water, a physiological saline solution, vegetable oil, a surfactant, a lipid, a solubilizing agent, a buffer, and a preservative.

<Regarding DNA of the Present Invention>

As one example of the “DNA encoding 23DOX of the present invention” included as an active ingredient of the composition, the sequence of cDNA encoding 23DOX derived from *S. chacoense* is shown in SEQ ID NO: 1. The amino acid sequence of the protein (Sc23DOX) encoded by the aforementioned sequence is shown in SEQ ID NO: 2. As another example of the “DNA encoding 23DOX of the present invention”, the sequence of cDNA encoding 23DOX derived from *S. lycopersicum* is shown in SEQ ID NO: 5. The amino acid sequence of the protein (S123DOX) encoded by the aforementioned sequence is shown in SEQ ID NO: 6. Note that, the S123DOX gene is located on the Chromosome 2, Solyc02g062460 of tomato. As another example of the “DNA encoding 23DOX of the present invention”, the sequence of cDNA encoding 23DOX derived from *S. tuberosum* is shown in SEQ ID NO: 3. The amino acid sequence of the protein (St23DOX) encoded by the aforementioned sequence is shown in SEQ ID NO: 4.

As one example of the “DNA encoding 23ACT of the present invention” included as an active ingredient of the composition, the sequence of cDNA encoding 23ACT derived from *S. chacoense* is shown in SEQ ID NO: 7. The amino acid sequence of the protein (Sc23ACT) encoded by the aforementioned sequence is shown in SEQ ID NO: 8. As another example of the “DNA encoding 23ACT of the

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present invention”, the sequence of cDNA encoding 23ACT derived from *S. lycopersicum* is shown in SEQ ID NO: 11. The amino acid sequence of the protein (S123ACT) encoded by the aforementioned sequence is shown in SEQ ID NO: 12. Note that, the S123ACT gene is located on the Chromosome 8, Solyc08g075210 of tomato. As another example of the “DNA encoding 23ACT of the present invention”, the sequence of cDNA encoding 23ACT derived from *S. tuberosum* is shown in SEQ ID NO: 9. The amino acid sequence of the protein (St23ACT) encoded by the aforementioned sequence is shown in SEQ ID NO: 10.

In the current state of the art, when persons skilled in the art obtain the sequence information on the “DNA encoding 23DOX of the present invention” or the “DNA encoding 23ACT of the present invention” (these two kinds of DNAs may be collectively referred to as “DNA (s) of the present invention”), they can make various modifications to its nucleotide sequence, and can produce a mutated gene that encodes a protein having an activity to introduce a hydroxy group to position 23 of a spirosolane skeleton or a protein having an activity to acetylate the hydroxy group of position 23 of the spirosolane skeleton. Moreover, nucleotide sequences can mutate in the natural world.

Therefore, the DNA encoding 23DOX of the present invention also includes DNA encoding a protein that consists of an amino acid sequence identical to the amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6 except that one or two or more amino acids are substituted, deleted, added, and/or inserted as long as it encodes a protein having an activity to introduce a hydroxy group to position 23 of a spirosolane skeleton. The DNA encoding 23ACT of the present invention also includes DNA encoding a protein that consists of an amino acid sequence identical to the amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12 except that one or two or more amino acids are substituted, deleted, added, and/or inserted as long as it encodes a protein having an activity to acetylate the hydroxy group of position 23 of the spirosolane skeleton.

Here, the “two or more” amino acids generally fall within 100 amino acids (e.g., within 90 amino acids, within 80 amino acids, and within 70 amino acids), preferably within 60 amino acids (e.g., within 50 amino acids, and within 40 amino acids), more preferably within 30 amino acids (e.g., within 20 amino acids, and within 10 amino acids), and particularly preferably within several amino acids (e.g., within 5 amino acids, within 3 amino acids, and within 2 amino acids) in the whole amino acid sequence of 23DOX or 23ACT.

Mutations can be introduced to each nucleotide sequence using a conventional methods such as the Kunkel method, the Gapped duplex method, or the like, or a method based on this method using, for example, a kit for mutation introduction (e.g., Mutant-K (manufactured by Takara Bio Inc.), Mutant-G (manufactured by Takara Bio Inc.)), or LA PCR in vitro Mutagenesis series kit (manufactured by Takara Bio Inc.), which uses the site-directed mutagenesis method.

In the current state of the art, when a specific DNA is obtained, persons skilled in the art can isolate a homologous gene encoding a protein having each activity from the same kind of plant or another kind of plant by using the sequence information on the DNA. Examples of plants for obtaining such a homologous gene include solanaceous plants. Specific examples thereof include: plants belonging to *Solanum* (e.g., *Solanum pandraeforme*, *Solanum verbascifolium*, *Solanum pennellii*, *Solanum aethiopicum*, *Solanum americanum*, *Solanum nigrum*, *Solanum carolinense*, *Solanum betaceum*, *Solanum lyratum*, *Solanum mammosum*, *Solanum*

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melongena, *Solanum muricatum*, *Solanum pseudocapsicum*, tomato (*Solanum lycopersicum*), and *Solanum chacoense*); plants belonging to *Capsicum* (e.g., *Capsicum annuum* (e.g., green pepper and paprika), *Capsicum baccatum*, *Capsicum cardenasii*, *Capsicum chinense*, *Capsicum frutescens*, and *Capsicum pubescens*); plants belonging to *Nicotiana* (e.g., *N. alata* and *Nicotiana* spp.); plants belonging to *Datura* (e.g., *Datura metel*, *Datura inoxia*, and *Datura stramonium*); plants belonging to *Brugmansia* (e.g., *Brugmansia arborea* and *Brugmansia suaveolens*); plants belonging to *Physalis* (e.g., *Physalis alkekengi* var. *franchetii*, and *Physalis philadelphica* (Tomatillo) (*Physalis ixocarpa*)); plants belonging to *Physalisstrum*; plants belonging to *Tubocapsicum*; plants belonging to *Petunia*; plants belonging to *Scopolia*; plants belonging to *Hyoscyamu*; plants belonging to *Atropa*; plants belonging to *Mandragora*; plants belonging to *Lycium*; and plants belonging to *Calibrachoa*.

Preferable examples of plants to obtain the homologous genes of the DNAs of the present invention include plants including 23 acetoxyl spirosolane. As such plants, for example, *Solanum nigrum* is known (Eich, Soloanaceae and Convolvulaceae: Secondary Metabolite (2008), Springer, Table 7.3). Preferable examples of plants to obtain the homologous gene of the “DNA encoding 23DOX of the present invention” include plants including 23 hydroxy spirosolane. As such plants, for example, *Solanum pandraeforme* and *Solanum verbascifolium* are known (Eich, Soloanaceae and Convolvulaceae: Secondary Metabolite (2008), Springer, Table 7.3).

Examples of a method for obtaining a homologous gene include the hybridization technique (Southern, E. M., J. Mol. Biol., 98: 503, 1975) and the polymerase chain reaction (PCR) technique (Saiki, R. K., et al. Science, 230: 1350-1354, 1985, Saiki, R. K. et al. Science, 239: 487-491, 1988). In order to isolate a homologous gene, the hybridization reaction is generally performed under stringent conditions. The stringent hybridization conditions are, for example, a condition of “1×SSC, 0.1% SDS, 37° C.” or other similar conditions, more stringent conditions are a condition of “0.5×SSC, 0.1% SDS, 42° C.” or other similar conditions, and still more stringent conditions are a condition of “0.2×SSC, 0.1% SDS, 65° C.” or other similar conditions. As the hybridization conditions are more stringent, isolation of DNA having a higher identity can be expected. Here, the above combinations of conditions including SSC, SDS, and temperature are examples, and required stringency can be achieved by appropriately combining, for example the concentration of DNA, the length of DNA, and the reaction time of hybridization.

There is no particular limitation as the “DNA encoding 23DOX of the present invention” and the “DNA encoding 23ACT of the present invention”, these are preferably a sequence having 80% or higher sequence identity, more preferably a sequence having 85% or higher sequence identity, still more preferably a sequence having 90% or higher (for example, 91% or higher, 92% or higher, 93% or higher, and 94% or higher) sequence identity, and particularly preferably 95% or higher (for example, 96% or higher, 97% or higher, 98% or higher, and 99% or higher) sequence identity, to the nucleotide sequence as set forth in SEQ ID NO: 1, 3, or 5 or the nucleotide sequence as set forth in SEQ ID NO: 7, 9, or 11. The sequence identity can be determined by using, for example, BLAST (Basic Local Alignment Search Tool at the National Center for Biological Information (for example, using default, i.e., using parameters of initial settings).

Therefore, the DNA encoding 23DOX of the present invention also includes DNA that hybridizes under stringent conditions with DNA consisting of a complementary sequence to a nucleotide sequence as set forth in SEQ ID NO: 1, 3, or 5 as long as the DNA encodes a protein having an activity to introduce a hydroxy group to position 23 of a spirosolane skeleton. Moreover, the DNA encoding 23ACT of the present invention includes DNA that hybridizes under stringent conditions with DNA consisting of a complementary sequence to a nucleotide sequence as set forth in SEQ ID NO: 7, 9, or 11 as long as the DNA encodes a protein having an activity to acetylate the hydroxy group of position 23 of the spirosolane skeleton. DNA including a sequence (degenerate sequence) based on degeneracy of genetic codes in each of the nucleotide sequences is also included in each DNA of the invention of the present application.

A protein encoded by the obtained homologous gene generally has a high identity to an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6 or an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12. The term "high identity" is, for example, 80% or higher sequence identity, preferably 85% or higher sequence identity, more preferably 90% or higher sequence identity (for example, 91% or higher sequence identity, 92% or higher sequence identity, 93% or higher sequence identity, and 94% or higher sequence identity), and still more preferably 95% or higher sequence identity (for example, 96% or higher sequence identity, 97% or higher sequence identity, 98% or higher sequence identity, and 99% or higher sequence identity). The sequence identity can be determined by using BLAST (Basic Local Alignment Search Tool at the National Center for Biological Information (for example, using default, i.e., using parameters of initial settings).

Therefore, the DNA encoding 23DOX of the present invention includes DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6, and has an activity to hydroxylate position 23 of a spirosolane skeleton. Moreover, the DNA encoding 23ACT of the present invention includes DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12, and has an activity to acetylate a hydroxy group of position 23 of a spirosolane skeleton.

Whether a protein encoded by DNA has an activity to introduce a hydroxy group to position 23 of a spirosolane skeleton or an activity to acetylate the hydroxy group of position 23 of the spirosolane skeleton can be determined in the following manner. For example, as described in Examples below, a protein encoding the DNA is synthesized using, for example, *E. coli*. In the presence of bivalent iron ions, ascorbic acid, and 2-oxoglutaric acid, a compound having a spirosolane skeleton (e.g., α-tomatine or 23 hydroxy tomatine) is added to the obtained protein, and the mixture is allowed to react. The obtained reaction product is analyzed by a method for analyzing glycoalkaloid using liquid chromatography of Matsuda et al. (Phytochem. Anal. 15: 121-124, 2004), Kozukue et al. (J. Agric. Food Chem. 52: 2079-2083, 2004), or Nakayasu et al. (Plant Physiol. 175: 120-133), which has been generally reported (for example, the reaction product is subjected to liquid chromatography, and the obtained fractions can be analyzed through mass spectrometry or with a UV or multiwavelength detector). Note that, persons skilled in the art can appropriately set analysis conditions.

The "DNA (s) of the present invention" are not particularly limited in terms of its form. Examples thereof include

genome DNA and chemically synthesized DNA in addition to cDNA. These DNAs can be prepared by using conventional methods known to persons skilled in the art. The genome DNA can be prepared in the following manner. Specifically, for example, genome DNA is extracted from a plant, to prepare a genomic library (as a vector, for example, a plasmid, a phage, a cosmid, BAC, or PAC can be used). This is developed, and is subjected to colony hybridization or plaque hybridization using a probe prepared based on a nucleotide sequence of the 23DOX gene (for example, DNA as set forth in SEQ ID NO: 1, 3, or 5) or the 23ACT gene (for example, DNA as set forth in SEQ ID NO: 7, 9, or 11). Primers specific to the 23DOX gene or the 23ACT gene are prepared, and are used to perform PCR, to thereby enable preparation of the genome DNA. Also, cDNA can be prepared in the following manner. Specifically, for example, cDNA is synthesized based on mRNA extracted from a plant. Then, the cDNA is inserted into a vector such as λZAP or the like, to prepare a cDNA library. Then, this is developed, and colony hybridization or plaque hybridization is performed in the same manner as the above method, or PCR is performed, to thereby enable preparation of the cDNA. In addition, a commercially available DNA synthesizer can be used to synthesize an intended DNA.

The DNA of the present invention may be included in the aforementioned compound in an aspect where the DNA is inserted into a vector. The vector is not particularly limited. Examples of the vector include vectors that can express an inserted DNA in a plant cell. The vector according to the present invention may include a promotor to express the DNA of the present invention constantly or inductively, and may appropriately include, for example, an enhancer, a terminator, and a selection marker.

When a transformed plant cell of the present invention is prepared by the below-described method via *Agrobacterium*, the DNA of the present invention may be included in the aforementioned compound in an aspect where the DNA is introduced to *Agrobacterium*.

More detailed aspects of the vector and *Agrobacterium* are exemplified in the following <Regarding plant cells of the present invention 1>.

<Regarding Plant Cells of the Present Invention 1>

Examples of the plant cell of the present invention that can regenerate a plant body having increased resistance against CPB include a transformed plant cell, which is obtained by introducing at least one DNA of the DNA encoding 23DOX of the present invention and the DNA encoding 23ACT of the present invention described above.

A plant from which plant cells of the present invention are derived is not particularly limited. Examples thereof include solanaceous plants such as potato and the like. The plant cell of the present invention is not limited to these plant species, but is particularly preferably general potato (*Solanum tuberosum*) that produces glycoalkaloid having a solanidine ring. Plants other than the general potato are not limited. Examples thereof include plant species, which form tubers and are included in the Potatoe subsection among *Solanum* species (Hawkes, The Potato (1990), Smithsonian Inst. Press, Table 6.1).

To a plant species that has low expression levels of the 23DOX gene and the 23ACT gene or that lacks these genes, both the DNA encoding 23DOX of the present invention and the DNA encoding 23ACT of the present invention are desirably introduced in order to increase resistance against CPB. On the other hand, as described in Examples below, some species of *S. tuberosum* may naturally have at least the Sc23ACT gene. To such plant species, at least the DNA

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encoding 23DOX of the present invention is desirably introduced. On the contrary, to a plant species that naturally have at least the 23DOX gene, at least the DNA encoding 23ACT of the present invention is desirably introduced.

The plant cell of the present invention may be a plant culture cell, the whole plant body of a cultivated plant, a plant organ (e.g., leaf, flower, stem, root, tuber, rhizome, or seed), or a plant tissue (e.g., epidermis, phloem, parenchyma, xylem, or vascular bundle). The plant cell further includes various forms of plant cells such as suspension cultured cells, a protoplast, a leaf slice, a callus, an immature embryo, a pollen, or the like.

Examples of a method for introducing the DNA of the present invention to a plant host include: the indirect introduction method such as the *Agrobacterium* infection method or the like; and the direct introduction method such as the electroporation method, the particle gun method, the polyethylene glycol method, the liposome method, the microinjection method, or the like.

For example, when the *Agrobacterium* infection method is used, a transformed plant cell to which the DNA of the present invention is introduced can be created in the following manner.

First, a recombinant vector for transformation is prepared, followed by transformation with *Agrobacterium*. The recombinant vector for transformation can be obtained in the following manner. Specifically, after a fragment including the DNA of the present invention is cleaved with an appropriate restriction enzyme, it is linked to an appropriate linker if necessary, and the resultant is introduced into a cloning vector for a plant cell. As a result, the recombinant vector for transformation can be obtained. As the cloning vector, a binary vector-based plasmid such as pBE2113Not, pBI2113Not, pBI2113, pBI101, pBI121, PGA482, pGAH, pBIG, or the like, or an intermediate vector-based plasmid such as pLGV23Neo, pNCAT, pMON200, or the like can be used.

When the binary vector-based plasmid is used, the DNA of the present invention is inserted between the border sequences (LB and RB) of the above binary vector, and this recombinant vector is amplified in *E. coli*. Then, the amplified recombinant vectors are introduced to, for example, *Agrobacterium tumefaciens* EHA105, C58, LBA4404, EHA101, or C58C1RifR by, for example, the electroporation method, and the *Agrobacterium* to which the DNA of the present invention is introduced may be used for transformation of a plant. In addition, the triparental mating method (Nucleic Acids Research, 12: 8711 (1984)) can be used to prepare *Agrobacterium* including the DNA of the present invention to be used for transformation. That is, *E. coli* that carries a plasmid including the DNA of the present invention, *E. coli* that carries a helper plasmid (e.g., pRK2013), and *Agrobacterium* are mixed and cultured, and are cultured on a culture medium including rifampicin and kanamycin. As a result, a zygote *Agrobacterium* for transformation can be obtained.

In order to express the DNA of the present invention that is a foreign gene in a plant cell, for example, a promoter, an enhancer, or a terminator for a plant is desirably linked before or after the present invention. Examples of an available promoter in the present invention include a 35S promoter derived from cauliflower mosaic virus (CaMV), a cone ubiquitin (UBI) promoter, a nopaline synthetase (NOS) gene promoter, and an octopine (OCT) synthetase gene promoter. As the enhancer, a virus-derived translational enhancer or a plant-derived translational enhancer can be used. Examples of the virus-derived translational enhancer

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include the sequences of tobacco mosaic virus, alfalfa mosaic virus RNA4, brome mosaic virus RNA3, potato virus X, tobacco etch virus, and other viruses. Examples of the plant-derived translational enhancer include the sequence derived from β -1,3 glucanase (Glu) of soybean and the sequence derived from a ferredoxin-binding subunit (PsaDb) of tobacco. As the terminator, for example, a CaMV-derived terminator or a NOS gene-derived terminator can be used. Note that, the promoter, the enhancer, and the terminator are not particularly limited to the above, and any promoter, any enhancer, and any terminator can be used as long as they are known to function in a plant body. These promoter, enhancer, and terminator are linked so that the DNA of the present invention to be expressed can function.

A promoter used when the DNA of the present invention is expressed in potato is not particularly limited. The promoter may be a promoter such as the 35S promoter or the like that can express a target gene in the whole plant, and may be a promoter derived from *S. chacoense* that can express a target gene in a site other than a tuber. Examples of the promoter include a promoter of the 23DOX gene or the 23ACT gene of *S. chacoense*. Persons skilled in the art can appropriately prepare these promoters by isolating several kilobases of the upstream site of the Sc23DOX coding region or the Sc23ACT coding region.

In order to efficiently select a target transformed plant cell, a selection marker gene is preferably used. Examples of the selection marker include the kanamycin-resistant gene (NP-TII), the hygromycin-resistant gene (htp), and the bialaphos-resistant genes (bar and pat). The DNA of the present invention and the selection marker gene may be incorporated into a single vector, or two kinds of recombinant DNAs, in which the DNA of the present invention and the selection marker gene are each incorporated to different vectors, may be used.

Moreover, when both the DNA encoding 23DOX of the present invention and the DNA encoding 23ACT of the present invention are introduced, these DNAs may be incorporated to a single vector, or two kinds of recombinant DNAs, in which the DNAs are each incorporated to different vectors, may be used, as described in Examples later.

As a method for introducing the DNA of the present invention to a plant host, gene insertion may be used by the genome editing method in addition to the indirect introduction method and the direct described above. The genome editing method is a method for modifying a target gene using site-specific nuclease (e.g., DNA double-strand cleavage enzyme such as Zinc finger nuclease (ZFN), transcription activator-like effector nuclease (TALEN), CRISPR-Cas9, or the like). Examples thereof include: methods using, for example, fusion proteins such as ZFNs (U.S. Pat. Nos. 6,265,196, 8,524,500, and 7,888,121, and European Patent No. 1720995), TALENs (U.S. Pat. Nos. 8,470,973 and 8,586,363), and PPR (pentatricopeptide repeat) (Nakamura et al., Plant Cell Physiol 53: 1171-1179 (2012)) fused with a nuclease domain or the like; and methods using a complex including guide RNA (gRNA) and a protein such as CRISPR-Cas9 (U.S. Pat. No. 8,697,359 and International Publication No. WO2013/176772), CRISPR-Cpf1 (Zetsche B. et al., Cell, 163 (3): 759-71, (2015)), Target-AID (K. Nishida et al., Targeted nucleotide editing using hybrid prokaryotic and vertebrate adaptive immune systems, Science, DOI: 10.1126/science.aaf8729, (2016)), or the like. <Regarding Plant Cells of the Present Invention 2>

As presented in the Examples later, *S. tuberosum* has the 23DOX gene and the 23ACT gene. However, *S. tuberosum* cannot sufficiently accumulate leptine because of their low

expression levels. That is, increased expression of these endogenous genes can increase the leptine accumulation amount and can increase resistance against CPB.

Therefore, the present invention also provides a transformed plant cell, which is capable of regenerating a plant body having increased resistance against CPB and has more increased expression of at least one endogenous DNA selected from the group consisting of the following (a) to (h) or has more increased expression of RNA corresponding to the DNA than an untransformed plant cell.

- (a) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6;
- (b) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6, and has an activity to hydroxylate position 23 of a spiro-solane skeleton;
- (c) DNA encoding a protein that consists of an amino acid sequence identical to the amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6 except that one or two or more amino acids are substituted, deleted, added, and/or inserted, and has the activity to hydroxylate the position 23 of the spirosolane skeleton;
- (d) DNA that hybridizes under stringent conditions with DNA consisting of a complementary sequence to a nucleotide sequence as set forth in SEQ ID NO: 1, 3, or 5, and encodes a protein having an activity to hydroxylate position 23 of a spirosolane skeleton;
- (e) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12;
- (f) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12, and has an activity to acetylate a hydroxy group of position 23 of a spirosolane skeleton;
- (g) DNA encoding a protein that consists of an amino acid sequence identical to the amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12 except that one or two or more amino acids are substituted, deleted, added, and/or inserted, and has an activity to acetylate a hydroxy group of the position 23 of the spirosolane skeleton; and
- (h) DNA that hybridizes under stringent conditions with DNA consisting of a complementary sequence to a nucleotide sequence as set forth in SEQ ID NO: 7, 9, or 11, and encodes a protein having an activity to acetylate a hydroxy group of position 23 of a spirosolane skeleton.

An increased rate of the expression compared to an untransformed plant cell is not particularly limited. The increase rate of the expression is preferably 1.1 times or higher, more preferably 1.2 times or higher, still more preferably 1.3 times or higher, and particularly preferably 1.5 times or higher.

The increase rate compared to the untransformed plant cell is calculated by measuring an expression level of at least one DNA of the DNA encoding 23DOX of the present invention and the DNA encoding 23ACT of the present invention or an expression level of RNA corresponding to those DNAs in the transformed plant cell and the untransformed plant cell through, for example, real-time PCR or semi-quantitative PCR using primers as set forth in SEQ ID NO: 30 and SEQ ID NO: 31.

Examples of a method for enhancing expression of an endogenous gene include a method for substituting a promoter upstream of each of the endogenous 23DOX gene and/or the endogenous 23ACT gene with another promoter

through, for example, genomic editing or homologous recombination. Examples of the "another promoter" include the 35S promoter from CaMV described above, the UBI promoter of corn, the NOS gene promoter, the OCT synthase gene promoter, the 23DOX gene promoter of *S. chacoense*, and the 23ACT gene promoter of *S. chacoense*. The "genome editing" as used herein is not particularly limited. Examples thereof include: SDN-2 (Site-Directed Nuclease 2, a type of genome editing in which a short DNA fragment 10 (template) homologous to a target sequence is artificially synthesized, and this is introduced in conjunction with an artificial restriction enzyme at the time of cleaving, to premeditatedly induce mutation of about one or several bases); and SDN-3 (Site-Directed Nuclease 3, a type of 15 genome editing in which a long DNA fragment, which includes about several thousand base pairs of genes (trans-gene) that can be crossed and are not derived from the same species or related species, is sandwiched and introduced with a homologous sequence, to form the DNA fragment at 20 a predetermined site on a genome). More specifically, expression of the endogenous 23DOX gene and the endogenous 23ACT gene can be increased by the following methods.

First, gRNA that recognizes 20 nucleotides including the 25 start codon site of the St23DOX gene, a Cas9 protein, and DNA linked with a St23DOX gene promoter, a 35S promoter derived from CaMV, and the St23DOX gene are introduced into a cell in a stem slice by the electroporation method. The complex of gRNA and Cas9 incorporated into 30 the cell cleaves the DNA between the St23DOX gene promoter and the St23DOX gene, and, at that time, the genome editing phenomenon SDN-3 occurs. As a result, the 35S promoter derived from CaMV can be inserted just upstream of the St23DOX gene. The stem slice obtained 35 after the treatment is subcultured in a plant hormone-containing MS culture medium (containing zeatin 2 ppm, indol-3-acetic acid 0.05 ppm, and agar 0.8%) at 25° C. under 16-hour illumination (photon flux density 32 μE/m²s)/8-hour non-illumination conditions every week, which makes 40 it possible to obtain a redifferentiated individual. DNA is extracted from the redifferentiated individual, and an individual into which the 35S promoter derived from CaMV is inserted is selected. The individual into which the 35S promoter derived from CaMV is inserted can increase an expression level of the endogenous St23DOX gene, which can produce leptinine. For the stem of the plant body, gRNA that recognizes 20 bases including the start codon site of the 45 St23ACT gene, a Cas9 protein, and DNA linked with a St23ACT gene promoter, a 35S promoter derived from CaMV, and the St23ACT gene are similarly introduced into the cell in the stem slice by the electroporation method. As a result, an expression level of the endogenous St23DOX gene and an expression level of the endogenous St23ACT gene can be increased, and therefore leptine can be produced.

Expression of the endogenous gene can also be increased by introducing DNA encoding a factor (transcriptional activator) that activates expression of the endogenous 23DOX gene and/or the endogenous 23ACT gene. In addition, the 50 expression of the endogenous gene can also be increased also by disrupting, through genome editing or the like, DNA encoding a factor (transcriptional repressor) that prevents expression of the endogenous 23DOX gene and/or the endogenous 23ACT gene. Persons skilled in the art can 55 increase expression of the endogenous 23DOX gene and/or the endogenous 23ACT gene by appropriately selecting transcriptional regulators in the group of glycoalkaloid bio-

synthesis gene, and targeting them based on, for example, cited references. For example, persons skilled in the art can easily increase expression of the endogenous gene by targeting JRE4 (see, Thagun et al., *Plant Cell Physiol.* 2016 57: 961-75), which is known as a transcription factor for the group of glycoalkaloid biosynthesis gene.

<Regarding Plant of the Present Invention and its Production Method 1>

The present invention provides a plant body regenerated from the plant cell. The regeneration of the plant body from the plant cell can be performed using conventional methods known to persons skilled in the art, depending on the type of cell. For example, a plant body can be regenerated from a transformed plant cell according to the method described in, for example, "Plant Cell Culture Manual", Yasuyuki Yamada (ed), KODANSHA SCIENTIFIC, 1984, and "Transformation Protocol [Botanical Edition]", Yutaka Tabei (ed), Kagaku-Dojin Publishing Co., Inc., published on Sep. 20, 2012.

Once a transformed plant to which the DNA of the present invention has been introduced in the genome or a plant in which the expression of the endogenous DNA has been increased is obtained, a progeny can be obtained from these plants through sexual reproduction or asexual reproduction. Therefore, the plant of the present invention includes generations and individuals that can be obtained by any means of cultivation or breeding based on the TO generation, such as progenies obtained from seeds of self-propagating or outcrossing plants, in addition to the current generation "TO generation" that is redifferentiated. In addition, propagating materials (e.g., seeds, fruits, cuttings, strains, calluses, and protoplasts) can be obtained from the plant body, its progeny, or clones, and then the plant body can be mass-produced based on them. Accordingly, the present invention includes a plant cell of the present invention, a plant including the cell, a progeny and a clone of the plant, and propagating materials of the plant, the progeny, and the clone.

The present invention also provides a method for producing a plant having increased resistance against CPB, the method including: introducing, to a plant cell, the DNA encoding 23DOX of the present invention and the DNA encoding 23ACT of the present invention; and regenerating a plant from a transformed plant cell to which the DNAs are introduced in the introducing. The "increased resistance against CPB" means that resistance against CPB is increased compared to a wild-type plant.

In, for example, the thus-obtained transformed plant to which the DNAs of the present disclosure were introduced and its next generation, whether the DNAs of the present invention are incorporated can be confirmed by extracting the DNAs from these cells and tissues using conventional methods and detecting the introduced DNAs of the present invention using conventional methods (e.g., the PCR method or the southern blotting method).

The present invention also provides a method for producing a plant having increased resistance against CPB, the method including: enhancing expression of an endogenous DNA encoding 23DOX of the present invention and an endogenous DNA encoding 23ACT of the present invention; and regenerating a plant from a plant cell having increased expression of the endogenous DNAs in the enhancing.

In, for example, the thus-obtained plant having increased expression of the endogenous DNAs and its next generation, whether expression of the DNAs is increased can be confirmed by extracting mRNA or a protein from these cells and tissues using conventional methods and detecting expression

of the DNAs of the present invention through detection of expression of the mRNA or the protein encoded by the DNA using conventional methods (e.g., the RT-PCR method, the northern blotting method, the ELISA method, or the western blot method).

In order to confirm whether the thus-obtained plant of the present invention has increased resistance against CPB, using CPB, the plant is investigated for the number of adult insects in a field, the number of insect damages, the adult insect consumption rate on a leaf cut in a disc shape, and the growth rate and the survival rate of larvae on a cut terrestrial part (see NPL 4), and the results can be compared to the results of a control (e.g., a plant to which the DNAs of the present invention are not introduced, a plant (e.g., wild-type) that has no increased expression of the endogenous DNAs of the present invention) to confirm that the resistance against CPB can be increased.

As described above, resistance against CPB depends on the accumulation amount of leptine. Thus, the amount of leptine is measured by an analysis method of glycoalkaloid using the aforementioned liquid chromatography, and, when the amount of leptine is greater than that of a control, it can be confirmed that resistance against CPB is increased.

<Regarding Determination Method of the Present Invention>

A method of the present invention for determining resistance against CPB in a plant includes: detecting, in a plant to be tested, presence or expression of the DNA encoding 23DOX of the present invention and the DNA encoding 23ACT of the present invention; and when the presence or expression of the DNAs is detected in the detecting, determining that the plant to be tested has resistance against CPB.

Detecting the "presence" of the DNAs of the present invention can be characterized by analyzing the DNAs in the plant to be tested or the nucleotide sequences in their expression controlling regions. Note that, the "DNA encoding 23DOX of the present invention" and the "DNA encoding 23ACT of the present invention" to be detected are described above.

For example, when the DNA encoding 23DOX of the present invention and the DNA encoding 23ACT of the present invention are not present on the genome DNA of a plant to be tested, it can be determined that the plant has no resistance against CPB. Even if the 23DOX gene and the 23ACT gene are present, CPB resistance is considered to be reduced or eliminated when a nucleotide is found to be inserted or deleted in these genes. Therefore, analysis of the DNA encoding the 23DOX of the invention and the nucleotide sequence of the 23ACT of the present invention can determine whether resistance against CPB is exhibited.

The nucleotide sequences of the regions controlling expression of the 23DOX gene and the 23ACT gene (enhancers, promoters, silencers, and insulators) can also be analyzed to determine whether resistance against CPB is exhibited.

In order to analyze nucleotide sequences of the 23DOX gene and the 23ACT gene or their expression control regions, amplification products obtained by amplifying these sequences through PCR can be used. When the PCR is performed, the primers used are not limited as long as they can each specifically amplify the genes or their expression control regions, and can be appropriately designed depending on their sequence information.

Here, a method for determining whether resistance against CPB is exhibited can include comparing with, for example, "nucleotide sequence of a control". Examples of the "nucleotide sequence of a control" to be compared to

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nucleotide sequences of the 23DOX gene and the 23ACT gene in a plant to be tested include nucleotide sequences encoding S123DOX, S123ACT, St23DOX, St23ACT, Sc23DOX, and Sc23ACT.

Comparison between the nucleotide sequences of the gene or its expression control region in a detected plant to be tested and the nucleotide sequence of the control can determine whether the plant to be tested has resistance against CPB. For example, when there is a significant difference in the nucleotide sequence compared to the nucleotide sequence of the control (particularly when the appearance of a new stop codon or frameshift results in significant changes in the molecular weight or the amino acid sequence of the encoded protein), it is determined that the plant to be tested is highly likely to be a plant having no resistance against CPB.

On the other hand, as shown in the examples described below, although *S. tuberosum* has the St23DOX gene and the St23ACT gene, the CPB resistance is not exhibited because the expression levels of these genes are low. Therefore, when it is compared to each of the expression control regions of the 23DOX gene and the 23ACT gene in a plant to be tested, each of the expression control regions of the S123DOX gene, the S123ACT gene, the Sc23DOX gene, and the Sc23ACT gene is used as a positive control nucleotide sequence, and each of the expression control regions of the St23DOX gene and the St23ACT gene is used as a negative control nucleotide sequence.

That is, when the nucleotide sequence of the expression control region in the detected plant to be tested has a high identity (for example, 90% or higher identity, preferably 95% or higher identity (96% or higher identity, 97% or higher identity, 98% or higher identity, 99% or higher identity; 100% identity)) with each of the expression control regions of the S123DOX gene, the S123ACT gene, the Sc23DOX gene, and the Sc23ACT gene, it is determined that the plant to be tested is highly likely to be a plant that has resistance against CPB. On the other hand, when the nucleotide sequence of the expression control region in the detected plant to be tested has a high identity (for example, 90% or higher identity, preferably 95% or higher identity (96% or higher identity, 97% or higher identity, 98% or higher identity, 99% or higher identity; 100% identity)) with each of the expression control regions of the St23DOX gene and the St23ACT gene, it is determined that the plant to be tested is unlikely to be a plant that has resistance against CPB.

As described above, even when some plants have the 23DOX gene and the 23ACT gene, such plants are unable to exhibit resistance against CPB due to their low expression levels. Therefore, it is desirable to combine the determination results using, as an indicator, the nucleotide sequences encoding the aforementioned 23DOX and the 23ACT with the determination results using, as an indicator, the nucleotide sequences of the expression control regions of the 23DOX gene and the 23ACT gene to determine whether the plant to be tested has resistance against CPB.

Detecting the "presence" of the DNAs of the invention can also be performed by detecting a DNA marker consisting of a DNA sequence that is a marker of the position where the DNA of the invention exists. As such a DNA marker, a DNA marker that is each present in, each adjacent to, or in the vicinity of the 23DOX gene and the 23ACT gene can be used.

The DNA marker available in the present invention is not particularly limited and a variety of generally known DNA markers can be suitably used. Examples thereof include

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microsatellite marker such as SSR (simple repeat sequence) marker, RFLP (restriction fragment length polymorphism) marker, SNP (single nucleotide polymorphism) marker, and the like.

In the determination method of the present invention, the DNA can be prepared from the plant to be tested using a conventional procedure, such as the CTAB method or the like. As a plant for preparing the DNA, not only a grown plant body but also a seed, a young plant body, and a tuber can be used. The nucleotide sequence can also be determined using a conventional procedure, such as the dideoxy method, the Maxam-Gilbert method, or the like. Commercial sequencing kits and sequencers may be used to determine base sequences.

Whether the nucleotide sequences of the 23DOX gene and the 23ACT gene or their expression control regions in the plant to be tested differ from the nucleotide sequences of the control can be analyzed indirectly by a variety of methods in addition to the direct determination of nucleotide sequences described above. Examples thereof include southern blotting, the PCR-SSCP (single-strand conformation polymorphism) method, the RFLP method and the PCR-RFLP method using restriction fragment length polymorphism (RFLP), the denaturant gradient gel electrophoresis (DGGE) method, the allele specific oligonucleotide (ASO) hybridization method, and the ribonuclease A mismatch cleavage method.

The determination method of the present invention is characterized by detecting expression of the DNA encoding 23DOX of the present invention and the DNA encoding 23ACT of the present invention in a plant to be tested.

The "detecting expression of DNA" used herein includes both detection at a transcriptional level and detection at a translational level. Furthermore, "detection of expression" means not only detection of presence or absence of expression but also detection of the extent of expression, and also includes detection of a molecular weight of an expression product of DNA.

Detection of the DNA of the present invention at a transcriptional level can be performed using a conventional procedure, such as the RT-PCR (Reverse transcribed-Polymerase chain reaction) method, the Northern blotting method, or the like. The primers used in performing the PCR are not particularly limited as long as the DNAs of the present invention can be specifically amplified, and can be appropriately designed based on the nucleotide sequences of the DNAs.

On the other hand, detection at a translational level can be performed using a conventional procedure, such as the Western blotting method, the ELISA method, or the like. Antibodies used in Western blotting may be polyclonal or monoclonal antibodies, and methods for preparing these antibodies are well known to persons skilled in the art.

As a result of detecting the expression, a plant to be tested is determined to have no resistance against CPB or to have low resistance against CPB when the expression level of the DNA of the present invention is significantly lower than that of a CPB-resistant plant (e.g., *S. chacoense*) or is lower than or equivalent to that of a CPB non-resistant plant (e.g., *S. tuberosum*) (for example, when the DNA of the invention is not substantially expressed), or a molecular weight of an expression product of the DNA of the present invention is significantly different from the molecular weight of a CPB-resistant plant.

<Regarding Plant of the Present Invention and its Production Method 2>

The present invention provides a method for producing a plant that has resistance against CPB using the determination method.

The production method includes: crossing a plant having resistance against Colorado potato beetle, with an arbitrary plant; determining the resistance against CPB in a plant obtained in the crossing, by the aforementioned method, and selecting a plant that is determined to have the resistance against CPB.

In addition, the method includes: crossing a plant having DNA encoding 23DOX of the present invention with a plant having DNA encoding 23ACT of the present invention; determining the resistance against CPB in a plant obtained in the crossing, by the aforementioned method; and selecting a plant that is determined to have the resistance against CPB.

The “plant having resistance against CPB” is not particularly limited as long as it has the resistance or an ability to biosynthesize leptine. Examples thereof include *S. chacoense*.

Examples of the “arbitrary plant” crossed with the variety include, but are not limited to, varieties having no resistance against CPB, and plants obtained by crossing plant varieties having resistance against CPB with varieties having no resistance against CPB. The term “arbitrary plant” may also have another property other than resistance against CPB. Example of the “another property” include, but are not limited to, resistance against pests other than CPB, resistance against various diseases, high yields, and early maturing.

The “plant having DNA encoding 23DOX of the present invention” may be any plant as long as it includes at least DNA encoding 23DOX. Examples thereof include plants having at least DNA encoding Sc23DOX: *S. chacoense*. The “plant having DNA encoding 23ACT of the present invention” may be any plant as long as it includes at least DNA encoding 23DOX. Examples thereof include plants having at least DNA encoding Sc23ACT: *S. chacoense* and *S. tuberosum* (e.g., Konafubuki, 97H32-6, Saikai 35, SAKURAFUBUKI, and pearlstarch).

In addition, use of the production method of the present invention enables selection of plants having resistance against CPB at, for example, a young plant stage, and enables cultivation of varieties having the characteristics in a short period of time. Therefore, the present invention also provides a method for breeding a plant having resistance against CPB.

The method includes: crossing a variety having resistance against CPB and with an arbitrary plant variety (plant line) or crossing a plant having DNA encoding 23DOX of the present invention with a plant having DNA encoding 23ACT of the present invention; and selecting presence or expression of the DNAs of the present invention as an indicator as described above, which therefore enables breeding. One example of a more specific selective breeding method includes a method including: crossing an arbitrary plant variety (e.g., *S. tuberosum*) with a plant variety having the DNA of the present invention (e.g., *S. chacoense*), to obtain a hybrid; backcrossing the obtained hybrid with the arbitrary plant variety; selecting a hybrid having the DNA of the present invention; and performing additional backcrossing. The backcrossing and the selecting are repeated several times, and preferably repeated 2 to 10 times. This method makes it possible to obtain a commercial variety having the DNA of the present invention.

The present invention also provides a plant that has resistance against CPB and is produced in this manner. The plant is not particularly limited as long as it has at least the DNA of the invention, but a substitution rate in the arbitrary plant variety on the whole chromosome is preferably 50% or more (e.g., 60% or more), more preferably 70% or more (e.g., 80% or more), and even more preferably 90% or more (e.g., 95% or more, 96% or more, 97% or more, 98% or more, and 99% or more). The substitution rate can be obtained by analyzing DNA marker present throughout the genome and calculating the rate in the arbitrary plant variety. Breeding through the DNA marker selection may also be performed according to, for example, Hamwieh et al. (2011) Euphytica, 179: 451-459.

<Regarding Kit Used in Determination Method of the Present Invention>

As described above, the presence or absence of resistance against CPB in a plant can be determined by detecting DNA encoding 23DOX and DNA encoding 23ACT of the present invention. Therefore, the present invention is an agent for determining resistance against CPB in a plant by the above-described determination method and includes at least one compound selected from the group consisting of (a) to (d) below:

- 25 (a) an oligonucleotide having a chain length of at least 15 nucleotides that hybridizes with a gene encoding the 23DOX of the present invention, a transcription product thereof, or a complementary nucleotide thereof;
- (b) an oligonucleotide having a chain length of at least 15 nucleotides that hybridizes with a gene encoding the 23ACT of the present invention, a transcription product thereof, or a complementary nucleotide thereof;
- (c) an antibody that binds to the 23DOX of the present invention; and
- (d) an antibody that binds to the 23ACT of the present invention.

The oligonucleotide according to the present invention may be in the form of a primer or in the form of a probe, in accordance with the above detection method.

40 The primers are not particularly limited as long as they hybridize with a gene encoding the 23DOX of the present invention or a gene encoding the 23ACT of the present invention (genome DNA), a transcription product thereof (mRNA) or a complementary nucleotide thereof (cDNA, CRNA), and can amplify and detect, for example, the transcription product. The primers may be only DNA, or part or all of the primers may be substituted with an artificial nucleic acid (modified nucleic acid), such as a cross-linked nucleic acid or the like. The size of the primers may be at 45 least about 15 nucleotides long, preferably 15 to 100 nucleotides long, more preferably 18 to 50 nucleotides long, and even more preferably 20 to 40 nucleotides long. Persons skilled in the art can design and produce such primers by conventional methods in accordance with the above detection methods.

50 The probe is not particularly limited as long as it hybridizes with a gene encoding the 23DOX of the present invention or to a gene encoding the 23ACT of the present invention, a transcription product thereof, or a complementary nucleotide thereof, and can detect them. The probe can be, for example, DNA, RNA, an artificial nucleic acid, or a chimeric molecule thereof. The probe can be either single-stranded or double-stranded. The size of the probe may be any size as long as it is at least about 15 nucleotides long, 55 and the size is preferably 15 to 1000 nucleotides long, more preferably 20 to 500 nucleotides long, and even more preferably 30 to 300 nucleotides long. Persons skilled in the

art can produce such probes by conventional methods. The probe may also be provided in the form of being immobilized on a substrate, such as a microarray or the like.

The antibody is not particularly limited as long as it can specifically bind to the 23DOX or the 23ACT of the present invention. For example, the antibody may be any of a polyclonal antibody and a monoclonal antibody, or may also be a functional fragment of the antibody (e.g., Fab, Fab', scFv). Persons skilled in the art can produce such antibodies by conventional methods. The antibody may also be provided in the form of being immobilized on a substrate, such as a plate or the like, for use in the ELISA method, antibody arrays, or the like.

The oligonucleotide or antibody contained in the kit of the present invention may also be labeled with a labeling substance depending on the detection method. Examples of the labeling substance include: fluorescent substances such as FITC, FAM, DEAC, R6G, TexRed, Cy5, and the like; enzymes such as β-D-glucosidase, luciferase, HRP, and the like; radioactive isotopes such as ³H, ¹⁴C, ³²P, ³⁵S, ¹²³I, and the like; affinity substances such as biotin, streptavidin, and the like; and luminescent materials such as luminol, luciferin, lucigenin, and the like.

Also, the oligonucleotide or antibody may be in the form that includes other components acceptable as a composition. Examples of the other components include carriers, excipients, disintegrators, buffers, emulsifiers, suspensions, stabilizers, preservatives, antiseptics, physiological saline, and secondary antibodies.

The kit of the present invention may also be combined with, for example, a substrate necessary for detecting a label, positive and negative controls, or a buffer used for diluting and washing samples, in addition to the above oligonucleotide and the antibody. In addition, the kit may include instructions of the kit.

Suitable embodiments of the present invention have been explained above, but the present invention shall not be construed as being limited to the above embodiments. For example, the plant cell, the plant, its production method of the present invention, the determination method of the present invention, and the plant production method using the determination method can be applied to achieve, for example, not only resistance against CPB but also production of leptine, like the composition of the present invention. That is, the present invention also provides the following aspects.

<12> A plant cell that is capable of regenerating a plant body having an increased accumulation amount of leptine, the plant cell including:

at least one DNA selected from the group consisting of (a) to (h) below, the at least one DNA being introduced to the plant cell,

(a) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6;

(b) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6, and has an activity to hydroxylate position 23 of a spirostanol skeleton;

(c) DNA encoding a protein that consists of an amino acid sequence identical to the amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6 except that one or two or more amino acids are substituted, deleted, added, and/or inserted, and has an activity to hydroxylate the position 23 of the spirostanol skeleton;

(d) DNA that hybridizes under stringent conditions with DNA consisting of a complementary sequence to a nucleotide sequence as set forth in SEQ ID NO: 1, 3, or 5, and encodes a protein having an activity to hydroxylate position 23 of a spirostanol skeleton;

(e) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12;

(f) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12, and has an activity to acetylate a hydroxy group of position 23 of a spirostanol skeleton;

(g) DNA encoding a protein that consists of an amino acid sequence identical to the amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12 except that one or two or more amino acids are substituted, deleted, added, and/or inserted, and has an activity to acetylate a hydroxy group of the position 23 of the spirostanol skeleton; and

(h) DNA that hybridizes under stringent conditions with DNA consisting of a complementary sequence to a nucleotide sequence as set forth in SEQ ID NO: 7, 9, or 11, and encodes a protein having an activity to acetylate a hydroxy group of position 23 of a spirostanol skeleton.

<13> A plant cell that is capable of regenerating a plant body having an increased accumulation amount of leptine,

wherein expression of at least one endogenous DNA selected from the group consisting of the (a) to (h) is increased.

<14> A plant that is regenerated from the plant cell according to <12> or <13> and has an increased accumulation amount of leptine.

<15> A method for producing a plant having an increased accumulation amount of leptine, the method including: introducing, to a plant cell, at least one DNA selected from the group consisting of the (a) to (h); and regenerating a plant from a transformed plant cell to which the DNA is introduced in the introducing.

<16> A method for determining an ability to generate leptine in a plant, the method including: detecting, in a plant to be tested, presence or expression of at least one DNA selected from the group consisting of the (a) to (d) and at least one DNA selected from the group consisting of the (e) to (h); and

when the presence or expression of the DNAs is detected in the detecting, determining that the plant to be tested has an ability to generate leptine.

<17> A method for producing a plant having an ability to generate leptine, the method including:

crossing a plant having an ability to generate leptine with an arbitrary plant;

determining, by the method according to <16>, the ability to generate leptine in an individual obtained in the crossing; and

selecting a plant that is determined to have the ability to generate leptine.

<18> A method for producing a plant having an ability to generate leptine, the method including:

crossing a plant having at least one DNA selected from the group consisting of the (a) to (d) with a plant having at least one DNA selected from the group consisting of the (e) to (h);

determining, by the method according to <16>, an ability to generate leptine in a plant obtained in the crossing; and selecting a plant that is determined to have the ability to generate leptine.

As described above, according to the present invention, a plant that has an ability to generate leptine can be obtained. Therefore, the present invention can be applied to, for example, organisms that are prevented from feeding by leptine, organisms that avoid leptine, organisms that are prevented from growing by leptine, and organisms that are killed by leptine, in addition to CPB.

All the references recited in the present specification are incorporated herein by reference as they are.

EXAMPLES

Hereinafter, the present invention will be described in detail by way of Examples, and the present invention shall not be construed as being limited to the following Examples.

It is revealed that the wild species potato *S. chacoense* exhibits resistance against CPB by accumulating leptines (leptine I and leptine II). Moreover, as shown in the lower part of FIG. 1, it is assumed that hydroxylation of position 23 of solanidanes (α -chaconine and α -solanine) produces leptinine I and leptinine II, followed by acetylation of the hydroxy groups of the position 23 of these compounds, to thereby accumulate leptines (see NPL 5). However, a gene that is involved with production of leptines and imparts resistance against CPB to *S. chacoense* has not been identified.

In order to identify the gene, the present inventors focused on the metabolic process (the upper part of FIG. 1) of a spirosolane glycoside (α -tomatine) in tomato (*S. lycopersicum*) that is the same solanaceous plant. The present inventors assumed that 23-position hydroxylase (also referred to as “23DOX”) and 23-position acetyltransferase (also referred to as “23ACT”) were involved with production of leptines in *S. chacoense*, similarly with the metabolic process of spirosolane glycoside in the tomato, and tried to identify genes encoding these enzymes in *S. chacoense* in the following manner. In tomato, the sequences of the genes encoding these enzymes have not been revealed. Therefore, they started to identify the sequences of the genes in tomato first.

(Example 1) Acquisition of 23DOX Gene

It is revealed that the metabolism of the spirosolane glycoside (production of esculeoside A from tomatine) in tomato is caused in the maturation process of the fruit. Therefore, the present inventors tried to identify a gene encoding hydroxylase (dioxygenase) expressed in the fruit of tomato.

Specifically, RNA was extracted from the fruit of dwarf tomato species Micro-Tom using an RNA extraction kit (product name: RNeasy, manufactured by QIAGEN), and cDNA was prepared using a cDNA synthesis kit for real-time PCR (product name: ReverTra Ace (Registered Trademark) qPCR RT Kit, manufactured by TOYOBO CO., LTD.).

On the other hand, the sequence (Solyc02g062460) encoding a protein having a structure of the dioxygenase specifically expressed in the fruit was found in the expression database of tomato (Sol Genomics Network: <https://solgenomics.net/>).

For the cDNA of the tomato fruit as a template, primers GGATCCATGGCATCTATAATCAG (SEQ ID NO: 13) and CTCGAGTCAAATACCAATAATCTTG (SEQ ID NO: 14), which were synthesized based on the aforementioned sequence, were used to perform PCR (30 cycles, manufactured by Takara Bio Inc., using Ex taq HS) at an annealing temperature of 55° C, to amplify the gene. This was cloned into a pMD19 vector (manufactured by Takara Bio Inc.), to obtain a gene fragment, followed by determining the full-length sequence (the protein encoded by this gene is also referred to as “S123DOX”). This amino acid sequence is shown in SEQ ID NO: 6.

Next, from a leaf of *S. chacoense* PI 458310 (seeds obtained from USDA Potato Genbank were seeded) that was expressing leptines, RNeasy (manufactured by QIAGEN) 15 was used to extract RNA, and a ReverTra Ace qPCR RT Kit (manufactured by TOYOBO CO., LTD.) was used to prepare cDNA. The exhaustive analysis of the nucleotide sequence of *S. chacoense* has not progressed, and most of the sequence remained unclear. Therefore, degenerate primers CTWAAACCAAACACTYCAYWATGGGAAT (SEQ ID NO: 15) and GGGTGTTYWTCATCYA-CWARTTCTTTGG (SEQ ID NO: 16), which were prepared based on the sequence of the S123DOX gene, were 20 used to perform PCR (30 cycles, manufactured by TOYOBO CO., LTD., using KOD FX Neo) at an annealing temperature of 55° C. The obtained PCR amplification product was cloned into a pCR4Blunt-TOPO vector (manufactured by Thermo Fisher Scientific), to obtain a gene fragment, followed by determining a partial sequence.

Then, the full-length ORF sequence of the cDNA fragments was determined by the RACE method. More specifically, a SMARTer RACE CDNA Amplification Kit (manufactured by Clontech Laboratories, Inc.) was used to synthesize cDNA for RACE from RNA of *S. chacoense* according to the protocol of the kit. For the cDNA for RACE 35 as a template, the universal primer affixed to the kit and the gene-specific primer TGGTGATTACCCTGAGGC-CAAAGA (SEQ ID NO: 17) in 5'-RACE, and the gene-specific primer GGTGATTGCATTCTCCTGTCCAC (SEQ ID NO: 18) and the universal primer affixed to the kit in 40 3'-RACE were each used to perform PCR (35 cycles, manufactured by Takara Bio Inc., using Ex Taq HS) at an annealing temperature of 58° C.

The amplified genes were cloned into a pMD19 vector (manufactured by Takara Bio Inc.) to analyze the sequences of gene fragments. As a result, a sequence expected to be a start codon was found in the fragment obtained through 45 5'-PACE, and a stop codon was found in the fragment obtained through 3'-RACE.

Then, for the cDNA of *S. chacoense*, primers CATATGG-CATCTACCAATCAGTTAAAGT (SEQ ID NO: 19) and GTCGACTCAAACACCGCAATAAGTCTTG (SEQ ID NO: 20), which were prepared based on the base sequence of the RACE fragment, were used to perform PCR (35 cycles, manufactured by Takara Bio Inc., using PrimeSTAR HS) at an annealing temperature of 55° C. The obtained PCR 55 amplification product was cloned into a pCR4Blunt-TOPO vector (manufactured by Thermo Fisher Scientific), to obtain a gene fragment, followed by determining the full-length sequence (the amino acid sequence encoded by the determined sequence is shown in SEQ ID NO: 2. The protein encoded by this gene is also referred to as “Sc23DOX”). Note that, S123DOX and Sc23DOX had a sequence identity of 87% in terms of an amino acid level.

(Example 2) Acquisition of 23ACT Gene

Like the above hydroxylase, the present inventors tried to identify a gene encoding acetyltransferase expressing in the

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fruit of tomato. Specifically, the sequence (Solyc08g075210) encoding a protein that has a structure of the acetyltransferase expressed in the fruit was found in the expression database of tomato (Sol Genomics Network: <https://solgenomics.net/>). For the cDNA of the tomato fruit, primers GGATCCCATATGACAGCAT-CAAGTTTGTATCTATG (SEQ ID NO: 21) and GTCGACCTAGAGATTGTAACGGAGAAGC (SEQ ID NO: 22), which were synthesized based on the aforementioned sequence, were used to perform PCR (40 cycles, manufactured by Takara Bio Inc., using PrimeStar HS) at an annealing temperature of 55°C., to amplify the gene. This was cloned into a pCR4Blunt-TOPO vector (manufactured by Thermo Fisher Scientific), to obtain a gene fragment, followed by determining the full-length sequence (hereinafter, a protein encoded by this gene is also referred to as "S123ACT". This amino acid sequence is shown in SEQ ID NO: 12).

RNA extracted from a leaf of *S. chacoense* was exhaustively sequenced using a next-generation sequencer to prepare EST (expressed sequence tag) database. In the EST database, S123DOX is used as a query sequence to perform blast, and a 3' fragment of a gene considered to encode the acetyltransferase was found.

Then, a 5' fragment of the gene was obtained, and the RACE method was performed in order to determine the full-length ORF sequence. The RACE method was performed using SMARTer RACE cDNA Amplification Kit (manufactured by Clontech Laboratories, Inc.). More specifically, according to the protocol of the kit, cDNA for RACE was synthesized from RNA of *S. chacoense*. For the cDNA for RACE as a template, the universal primer affixed to the kit and the gene-specific primer TGCCATC-CACTGGCATTACATGG (SEQ ID NO: 23) were used to perform PCR (35 cycles, manufactured by Takara Bio Inc., using Ex Taq HS) at an annealing temperature of 58°C., to amplify the gene. Then, the obtained amplification product was cloned into a pMD19 vector (manufactured by Takara Bio Inc.), followed by sequence analysis of the gene fragment. As a result, a sequence expected to be a start codon was found in the fragment obtained by 5'-RACE.

In order to determine the full-length ORF sequence of a gene that was considered to encode acetyltransferase, using cDNA of *S. chacoense* as a template, primers CATATGGCAGCATCAAGTGTAT (SEQ ID NO: 24) and GTCGACTTAATTAAGATTAGTAATGGAGAAGA (SEQ ID NO: 25), which were prepared based on the base sequences of the fragments, were used to perform PCR (30 cycles, manufactured by Takara Bio Inc., using PrimeStar) at an annealing temperature of 55°C., to amplify the gene. The obtained PCR product was cloned into a pENTR/D-TOPO vector (manufactured by Thermo Fisher Scientific) to obtain a gene fragment, followed by determining the full-length sequence (Sc23ACT, its amino acid sequence is shown in SEQ ID NO: 8.). Note that, S123ACT and Sc23ACT had an amino acid same homology of 30%.

(Example 3) Detection of In Vitro Enzyme Activity of 23DOX

Whether the protein encoded by the gene identified in Example 1 was able to introduce a hydroxy group to position 23 of these compounds using α-tomatine or α-solanine as a substrate as assumed was examined in the following manner.

First, in order to synthesize 23DOX in *E. coli*, the S123DOX gene and the Sc23DOX gene were each linked to a pCold ProS2 vector (manufactured by Takara Bio Inc.),

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and were introduced into *E. coli* BL21 (DE3). The obtained recombinant *E. coli* was cultured at 37°C. until the OD₆₀₀ value reached 0.5, was cooled to 15°C., and was left to stand for 30 minutes. IPTG was added thereto so that the final concentration reached 0.1 mM, and the mixture was cultured under shaking at 15°C. for 24 hours, to induce expression of the recombinant protein.

Then, the *E. coli* bodies whose expression was induced were collected, and were suspended in a sonication buffer [50 mM Bis-Tris-HCl (pH 7.0), 150 mM NaCl, 1 mM dithiothreitol, 10% glycerol]. After an ultrasonic crushing machine was used to crush bacterial cells, the mixture was subjected to centrifugal separation, and crude extraction fractions were obtained from the supernatant. A 100 mM Bis-Tris-HCl solution (pH 7.2, containing 5 mM 2-oxoglutaric acid, 10 mM sodium ascorbate, and 200 μM FeSO₄) containing a 50 μM substrate was added to the mixture, and was allowed to react.

The obtained reaction product was analyzed through LC-MS (manufactured by Waters, product name: UPLC-ESI-MS ACQUITY). As the column, ACQUITY HSS T3 1.8 μm φ 2.1×100 mm (manufactured by Waters) was used. In LC analysis, gradient elution was performed using a mobile phase (A: 0.1% formic acid water and B: acetonitrile). The gradient elution was performed under the condition: maintaining for from 0 to 3 minutes, 90% A/10% B; 3 to 33 minutes, 90% A/10% B to 57.5% A/42.5% B; 33 to 33 minutes, 57.5% A/42.5% B to 0% A/100% B; and maintaining for from 38 to 43 minutes, 100% B.

As a result, as shown in FIG. 2 and FIG. 3, it was revealed that a product to which a hydroxy group was introduced at position 23 was obtained not only in those derived from *S. lycopersicum* (S123DOX) but also in 23DOX (Sc23DOX) derived from *S. chacoense* when α-tomatine was a substrate.

However, a new reaction product was not obtained in both S123DOX and Sc23DOX when α-solanine was a substrate (not illustrated in the figures).

(Example 4) Detection of In Vitro Enzyme Activity of 23ACT

Whether the protein encoded by the gene identified in Example 2 was able to acetylate a hydroxy group of position 23 of these compounds using 23 hydroxy tomatine, leptinone I, or leptinone II as a substrate as assumed was examined in the following manner.

First, in order to synthesize 23ACT in vitro, the S123ACT gene and the Sc23ACT gene were each linked to a pCold ProS2 (manufactured by Takara Bio Inc.), and were introduced into *E. coli* BL21 (DE3). The obtained recombinant *E. coli* was cultured at 37°C. until the OD₆₀₀ value reached 0.5, was cooled to 15°C., and was left to stand for 30 minutes. IPTG was added thereto so that the final concentration reached 0.1 mM, and the mixture was cultured under shaking at 15°C. for 24 hours, to induce expression of the recombinant protein. Then, the *E. coli* bodies whose expression was induced were collected, and were suspended in a sonication buffer [50 mM Bis-Tris-HCl (pH 7.0), 150 mM NaCl, 1 mM dithiothreitol, 10% glycerol]. After an ultrasonic crushing machine was used to crush bacterial cells, the mixture was subjected to centrifugal separation, and crude extraction fractions were obtained from the supernatant. A 100 mM Bis-Tris-HCl solution (pH 7.2, containing 400 μM acetyl COA) containing a 50 μM substrate was added to the mixture, and was allowed to react.

The obtained reaction product was analyzed through LC-MS (manufactured by Waters, product name: UPLC-

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ESI-MS ACQUITY). As the column, ACQUITY HSS T3 (1.8 µm φ2.1×100 mm) (manufactured by Waters) was used. In LC analysis, gradient elution was performed using a mobile phase (A: 0.1% formic acid water and B: acetonitrile). The gradient elution was performed under the condition: 0 to 30 minutes, 90% A/10% B to 45% A/55% B; 30 to 31 minutes, 45% A/55% B to 100% B; and maintaining for from 31 to 35 minutes, 100% B.

As a result, as shown in FIG. 4 and FIG. 5, it was revealed that a product to which an acetoxy group was introduced at position 23 was obtained in the presence of S123ACT or Sc23ACT when 23 hydroxy tomatine was a substrate.

On the other hand, a new reaction product was not obtained in both S123ACT and Sc23ACT when leptinine I or leptinine II was a substrate (not illustrated in the figures).

As described above, it was revealed that 23DOX and 23ACT of tomato and *S. chacoense* encoded by the genes identified this time catalyze hydroxylation reaction and acetylation reaction at position 23 of α-tomatine (spirosolane skeleton). On the other hand, the involvement of these enzymes was not observed in the previously assumed reaction process from α-solanine (solanidine skeleton) to leptine. That is, it was suggested that 23DOX and 23ACT are enzymes that specifically act on a compound having a spirosolane skeleton.

(Example 5) Analysis of Steroid Glycoalkaloid in Potato to which 23DOX Gene is Introduced

A transformed potato to which the 23DOX gene derived from *S. chacoense* was introduced was created. Then, whether leptinine I and leptinine II were produced in this transformed body was examined in the following manner.

First, the Sc23DOX gene was linked to a pRI201 vector (manufactured by Takara Bio Inc.) to prepare a pRI201_Sc23DOX vector (see the upper part of FIG. 6). This was introduced into *Agrobacterium tumefaciens* EHA105 strain. The vector-containing *Agrobacterium tumefaciens* was cultured under shaking at 28°C. for 12 hours in YBS liquid culture medium [5 g/L beef extract, 1 g/L yeast extract, 5 g/L peptone, 5 g/L sucrose, 2 mM magnesium sulfate (pH 7.2)] containing 50 ppm kanamycin. After the culture solution (1.5 mL) was subjected to centrifugation at 10,000 rpm for 3 minutes to collect bacterial cells, the bacterial cells were suspended again in MS culture medium [Murashige & Skoog, see Physiol. Plant., 15, 473-497 (1962)] containing 1.5 mL of 3% sucrose. This was used as a bacterial liquid for infection.

The stems of the potato (*Solanum tuberosum*) variety "Sassy" cultivated in vitro, which had been cut into 3 to 5 mm pieces without joints, were used as a material for *Agrobacterium* infection. After being immersed in the above bacterial liquid of *Agrobacterium*, this was placed on sterilized filter paper to remove excessive *Agrobacterium*. This was placed on a plant hormone-containing MS culture medium (containing acetosyringone 100 µM, zeatin 2 ppm, indole-3-acetic acid 0.05 ppm, and agar 0.8%) in a petri dish, and was cultured for 3 days. The culture was performed at 25°C. for 16 hours under the condition: 16-hour illumination (photon flux density 32 HE/m²s)/8-hour non-illumination. Then, it was subcultured every two weeks in a culture medium containing 250 ppm of carbenicillin instead of acetosyringone. As a result, a redifferentiated individual was able to be obtained.

About 100 mg of a leaf of the obtained redifferentiated individual (#9) was frozen with liquid nitrogen, and was crushed with a mixer mill (1/30 sec, 2 min). Then, 300 µL of

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methanol was added to the crushed leaf, and the mixture was sonicated for 10 minutes. The resultant was subjected to centrifugal separation (15,000 rpm, 10 min), and the supernatant was collected. This extraction operation was repeated three times, the collected supernatant was dried under reduced pressure, and the residues were dissolved again in 200 µL of methanol. Then, 20 µL of re-dissolved solution was dissolved in 180 µL of methanol, and glycoalkaloid was analyzed through LC-MS (manufactured by Waters, product name: UPLC-ESI-MS ACQUITY). As the column, ACQUITY HSS T3 (1.8 µm φ2.1×100 mm) (manufactured by Waters) was used. In LC analysis, gradient elution was performed using a mobile phase (A: 0.1% formic acid water and B: acetonitrile). The gradient elution was performed under the condition: 0 to 30 minutes, 90% A/10% B to 45% A/55% B; 30 to 31 minutes, 45% A/55% B to 100% B; and maintaining for from 31 to 35 minutes, 100% B. The resultant was compared with a sample containing leptinine, which was a flower extract of *S. chacoense* PI 458310.

As a result, as shown in FIG. 7 and FIG. 8, it was revealed that leptinine I and leptinine II can be produced by expression of Sc23DOX in *S. tuberosum* that has not been recognized to produce leptinine.

(Example 6) Analysis of Steroid Glycoalkaloid in Potato Hairy Roots to which 23DOX Gene and 23ACT Gene were Introduced

Then, a transformed potato to which the 23DOX gene and the 23ACT gene derived from *S. chacoense* were introduced was created. Whether leptine I and leptine II were produced in this transformed body was examined in the following manner.

First, both the Sc23DOX gene and the Sc23ACT gene were linked to pBin+201 to produce a vector pBin+201_Sc23DOX Sc23ACT (see the lower part of FIG. 6), and this was introduced into *Agrobacterium Rhizogenes* C15834 strain. The vector-containing *Agrobacterium* was cultured under shaking at 28°C. for 12 hours in a 50 ppm kanamycin-containing YEB liquid culture medium [5 g/L beef extract, 1 g/L yeast extract, 5 g/L peptone, 5 g/L sucrose, 2 mM magnesium sulfate (pH 7.2)]. The culture solution was spread on YEB agar culture medium (2% agarose), and was cultured at 28°C. for 72 hours in a dark place. The potato variety "Sassy" cultured in vitro was cut into 1 to 1.5 cm pieces, and the tip of the root side of the stem was attached to the colony of *Rhizogenes*, and was stuck to B5 culture medium (containing 0.3% Gelrite and 2% sucrose) in a plant box so that the tip at the root side faced upward. This was cultured at 20°C. for 20 days in a dark place. The upper part of the stem in which formation of hairy roots had been confirmed was cut and was transferred to MS culture medium (containing 0.3% Gelrite, 2% sucrose, and 250 ppm Cefotaxime). The upper part of the stem was cultured at 25°C. for 7 days in a dark place and was sterilized. The tip (1 cm) of the grown hairy roots was cut, was transferred to B5 culture medium (containing 0.3% Gelrite, 2% sucrose, and 250 ppm Cefotaxime), and was cultured at 25°C. for 7 days in a dark place. The obtained hairy roots were cut into slices, the slices were transferred to B5 liquid culture medium (containing 2% sucrose), and were further cultured under shaking (100 rpm) at 20°C. for 14 days in a dark place. Then, 100 mg of products that proliferated from the slices were frozen with liquid nitrogen, and were crushed with a mixer mill (1/30 sec, 2 min). Then, 300 µL of methanol was added to the crushed products and the mixture was sonicated for 10 minutes. The mixture was subjected to centrifugal

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separation (15,000 rpm, 10 min), and the supernatant was collected. This extraction operation was repeated three times, the collected supernatant was dried under reduced pressure, and the residues were dissolved again in 200 µL of methanol. Then, 20 µL of the re-dissolved solution was dissolved in 180 µL of methanol, and glycoalkaloid was analyzed through LC-MS (manufactured by Waters, product name: UPLC-ESI-MS ACQUITY). As the column, ACQUITY BEH C-18 1.7 µm φ 2.1×100 mm (manufactured by Waters) was used. In LC analysis, gradient elution was performed using a mobile phase (A: 0.1% formic acid water and B: acetonitrile). The gradient elution was performed under the condition: maintaining for from 0 to 3 minutes, 90% A/10% B; 3 to 33 minutes, 90% A/10% B to 57.5% A/42.5% B; 33 to 38 minutes, 57.5% A/42.5% B to 0% A/100% B; and maintaining for from 38 to 43 minutes, 100% B. The resultant was compared with a sample containing leptine, which was a flower extract of *S. chacoense* PI 458310.

As a result, as shown in FIG. 9 and FIG. 10, it was revealed that leptine I and leptine II can be produced by expression of Sc23DOX and Sc23ACT in *S. tuberosum* that has not been recognized to produce leptine.

As described above, it was revealed that, in *S. chacoense*, leptine is not produced from a compound (solanine, chaconine) having a solanidine skeleton as assumed previously, and leptine is produced by introducing a hydroxy group and then an acetoxy group to position 23 of a compound having a spirosolane skeleton, followed by converting the spirosolane skeleton of the compound into a solanidine skeleton.

(Example 7) Assay Regarding Presence or Absence of Sc23DOX Gene in Potato Varieties or Lines Used for Potato Breeding

In order to confirm that *S. tuberosum* that does not exhibit resistance against CPB has no Sc23DOX gene, the following analysis was performed.

From potato varieties that exhibit no resistance against CPB or lines used for potato breeding ("Irish Cobbler potato", "May queen", "Sayaka", "Sassy", "Konafubuki", "Desiree", "97H32-6", "Saikai 35", "Hokkai 87", "VTn 62-33-3", and "VTn 62-33-3") and a wild species that exhibits resistance against CPB ("*S. chacoense* PI 458310"), DNAs were extracted by the CTAB method (Hosaka and Hanneman Euphytica, 1998, 103: 265-271). For the extracted DNA, a primer GGCATCTACCAAATCAGTTAAAG (SEQ ID NO: 26) and a primer GTCTTGAAAA-CATCACTGGGAG (SEQ ID NO: 27) were used to perform PCR (35 cycles, manufactured by BioLine, using BIOTAQ) at an annealing temperature of 60° C., to amplify the gene. The obtained results were shown in FIG. 11.

As a result, about 1,700 bases of amplified fragments were found in *S. chacoense* PI 458310. In addition, amplified fragments were found even in Konafubuki and Saikai 35 that use *S. chacoense* in its breeding process. However, these amplified fragments were about 2,000 bases in size, which were larger than that of *S. chacoense* by about 200 bases. As a result of determining these base sequences, it was able to be confirmed that they have different sequences from that of the Sc23DOX gene (for this sequence, see Example 10).

Therefore, within the examined scope, one having the Sc23DOX gene was not detected in materials used in the aforementioned varieties or breeding in addition to a CPB-resistant potato, *S. chacoense* PI 458310.

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(Example 8) Assay Regarding Presence or Absence of Sc23ACT Gene in Potato Varieties or Lines Used for Potato Breeding

5 In order to confirm that *S. tuberosum* that does not exhibit resistance against CPB has no Sc23ACT gene, the following analysis was performed in the same manner as in Example 7.

10 From potato varieties or lines used for potato breeding ("Irish Cobbler potato", "May queen", "Sayaka", "Sassy", "Konafubuki", "Desiree", "97H32-6", "Saikai 35", "Hokkai 87", "VTn 62-33-3", "W553-4", and "*S. chacoense* PI 458310"), DNAs were extracted by the CTAB method. For the extracted DNA, a primer GATTATGAATTTCATAATTTG (SEQ ID NO: 28) and a primer TACAGGTAGTGTGACAACGAGGATC (SEQ ID NO: 29) were used to perform PCR (40 cycles, manufactured by BioLine, using BIOTAQ) at an annealing temperature of 60° C., to amplify the gene. The obtained results were shown in FIG. 12.

15 As a result, it was surprisingly revealed that Konafubuki, 97H32-6, and Saikai 35 that exhibit no resistance against CPB also have the Sc23ACT gene. Konafubuki, 97H32-6, and Saikai 35 use *S. chacoense* (W84 and che525-3) in its breeding process (Asama et al., Bulletin of the Hokkaido Prefectural Agricultural Experiment Station, 1982, 48: 75-84, Phumichai et al., Genome, 2005, 48: 977-984). There is no report that W84 and che525-3 accumulate leptine. However, these potatoes can be expected to exhibit an ability 20 to generate leptine by introducing the Sc23DOX gene to Konafubuki and 97H32-6, which has been shown to have the Sc23ACT gene.

25 Therefore, it was found that detection of the Sc23ACT gene can easily reveal materials that potentially have such an ability to generate leptine

30 SAKURAFUBUKI and pearlstarch, which use Konafubuki in the breeding process, were also analyzed. As a result, it was revealed that these potatoes have Sc23ACT (not illustrated in the figures).

(Example 9) Analysis of Steroid Glycoalkaloid and Genes in Progeny Obtained by Crossing *S. chacoense* with Potato

40 In order to confirm that the Sc23DOX gene and the Sc23ACT gene correlate with accumulation of leptine, the following analysis was performed.

45 First, five lines of seedling were obtained from *S. chacoense* PI 458310 expressing leptine. It was confirmed that all of them accumulated leptine.

50 Next, the five lines, and a potato line 97H32-6 expressing no leptine were crossbred to obtain a hybrid seed. Then, from leaves of 263 individuals obtained by growing the hybrid seeds, DNA was extracted by the method of Hattori et al. (Breed. Sci. 57: 305-314), and the Sc23DOX gene and the Sc23ACT gene were each amplified by the methods described in Examples 7 and 8. From about 100 mg of leaves obtained by growing the hybrid seeds, each steroid glycoalkaloid was analyzed by the method described in Example 3. The obtained results are shown in Table 1. The LC-MS analysis data of three lines are shown in FIG. 13.

TABLE 1

Line numbers	Detection of leptine
PI 458310-2 × 97H32-6 3	Detected
PI 458310-2 × 97H32-6 19	Detected

TABLE 1-continued

Line numbers	Detection of leptine
PI 458310-2 × 97H32-6 32	Detected
PI 458310-2 × 97H32-6 34	Detected
PI 458310-2 × 97H32-6 44	Detected
PI 458310-2 × 97H32-6 57	Detected
PI 458310-2 × 97H32-6 62	Detected
PI 458310-2 × 97H32-6 67	Detected
PI 458310-2 × 97H32-6 98	Detected
PI 458310-2 × 97H32-6 113	Detected
PI 458310-3 × 97H32-6 2	Detected
PI 458310-3 × 97H32-6 17	Detected
PI 458310-3 × 97H32-6 19	Detected
PI 458310-3 × 97H32-6 22	Detected
PI 458310-3 × 97H32-6 37	Detected
PI 458310-6 × 97H32-6 3	Detected
PI 458310-6 × 97H32-6 4	Detected
PI 458310-6 × 97H32-6 17	Detected
PI 458310-6 × 97H32-6 26	Detected
PI 458310-6 × 97H32-6 36	Detected

As a result, all of the hybrid individuals were found to have the Sc23DOX gene and the Sc23ACT gene. This revealed that the seedling individual of *S. chacoense* PI 458310 homozygously has the Sc23DOX gene, and that the seedling individual of *S. chacoense* PI 458310, the potato line 97H32-6, or both homozygously have the Sc23ACT gene.

As shown in Table 1, all of the hybrid individuals were found to accumulate leptine. Therefore, it was confirmed that presence of the Sc23DOX gene and the Sc23ACT gene correlates with accumulation of leptine.

Moreover, 97H32-6 can be backcrossed with the line obtained in the above manner, which has the Sc23DOX gene and the Sc23ACT gene and produces leptine, to obtain a hybrid seed. A hybrid seed that produces leptine can be determined by analysis of presence or absence of these genes in the same manner as the above.

(Example 10) Verification of 23DOX Gene in *S. tuberosum*

In tblast analysis targeted for the sequences in the potato (*S. tuberosum*) genome database (Spud DB: <http://solanaceae.plantbiology.msu.edu/index.shtml>), even the sequence (PGSC0003DMT400023800) having the highest homology to Sc23DOX has a low identity of 79%, and it has been believed that no 23DOX gene exists in *S. tuberosum*.

Surprisingly, however, as shown in Example 7, amplification of a fragment of about 2,000 bases, which was about 200 bases larger than the amplification product (about 1,700 bases) derived from the Sc23DOX gene of *S. chacoense* PI 458310, was found in *S. tuberosum*. As a result of determining the base sequences, it was revealed that the sequence extremely close to the Sc23DOX gene was amplified.

For the cDNA of *S. tuberosum* variety, Sassy, a primer CACCATGGCATCTACCAAATCAGTTAAAG (SEQ ID NO: 30) and a primer TCAAACACCGCAATAAGTCTT-GAAA (SEQ ID NO: 31) were used to perform PCR (40 cycles, manufactured by Takara Bio Inc., using PrimeSTAR) at an annealing temperature of 55°C. The obtained PCR amplification product was cloned into a pENTR/D-TOPO vector (manufactured by Thermo Fisher Scientific), to obtain a gene fragment, followed by determining the full-length sequence (the amino acid sequence encoded by the determined nucleotide sequence is shown in SEQ ID NO: 4. The protein encoded by this gene is also referred to as "St23DOX" hereinafter.). Note that, St23DOX and

Sc23DOX had a sequence identity of 94% in terms of an amino acid level. As a result of further analysis, it was found that at least Konafubuki, SAKURAFUBUKI, pearlstarch, Saikai 35, and Irish Cobbler potato in addition to Sassy also have the St23DOX gene.

(Example 11) Detection of In Vitro Enzyme Activity of St23DOX

- 10 The St23DOX gene identified in Example 10 was analyzed in the same method described in Example 3. As a result, as shown in FIGS. 2 and 3, it was revealed that, a product to which a hydroxy group was introduced at position 23 can be obtained even in those derived from *S. tuberosum* (St23DOX) when α-tomatine was a substrate. That is, it was revealed that St23DOX can be involved with production of leptinines similarly with Sc23DOX and S123DOX. On the other hand, in *S. tuberosum* (variety: Sassy), the resistance against CPB via production of leptinine is not observed.
15 Therefore, the above suggests that St23DOX is not expressed enough to contribute to production of leptinine at least in variety Sassy.

(Example 12) Verification of 23ACT Gene in *S. tuberosum*

In tblast analysis targeted for the sequences in the potato (*S. tuberosum*) genome database (Spud DB: <http://solanaceae.plantbiology.msu.edu/index.shtml>), even the sequence (PGSC0003DMT400023800) having the highest homology to Sc23ACT has a low identity of 75%, and it has been confirmed that excessive 30 amino acids were added to the N-terminal. Therefore, it was believed that one exceeding a same homology of 50% was not found in one having the full length similar to that of Sc23ACT, and no 23ACT gene exists in *S. tuberosum*.

Surprisingly, however, it was suggested that the sequence extremely close to the Sc23ACT gene was found also in *S. tuberosum* when the assay primer was prepared in Example 40 8. For the genome of the variety Sassy, a primer CATATGGCAGCATCAAGTTGTGT (SEQ ID NO: 32) and a primer GTCGACTTAATTAGATTAGTAATTG-GAGAAG (SEQ ID NO: 33) were used to perform PCR (40 cycles, manufactured by Takara Bio Inc., using PrimeSTAR HS) at an annealing temperature of 55°C. The obtained PCR amplification product was cloned into a pCR4Blunt-TOPO vector (manufactured by Thermo Fisher Scientific), to obtain a gene fragment, followed by determining the full-length sequence (the amino acid sequence encoded by the determined nucleotide sequence is shown in SEQ ID NO: 10. The protein encoded by this gene is also referred to as "St23ACT"). Note that, St23ACT and Sc23ACT had a sequence identity of 91% in terms of an amino acid level.

(Example 13) Detection of In Vitro Enzyme Activity of St23ACT

The St23ACT gene identified in Example 12 was analyzed in the same method described in Example 4. As a result, as shown in FIG. 4 and FIG. 5, it was revealed that a product to which an acetoxy group was introduced at position 23 was obtained in the presence of St23ACT when 60 23 hydroxy tomatine was a substrate. That is, it was revealed that St23ACT can be involved with production of leptinines similarly with Sc23ACT and S123ACT. On the other hand, in *S. tuberosum* (variety: Sassy), the resistance against CPB via accumulation of leptine is not observed. Therefore, the

above suggests that St23ACT is not expressed enough to contribute to production of leptine, at least in variety Sassy, similarly with the above St23DOX.

(Example 14) Introduction of Sc23DOX Gene to Konafubuki Having Sc23ACT Gene

It was revealed in Example 8 that Konafubuki has the sequence of Sc23ACT. In order to confirm that this sequence functions and Konafubuki expresses 23ACT activity, steroid glycoalkaloid in potato hairy roots of Konafubuki, to which the Sc23DOX gene had been introduced, was analyzed.

Specifically, in the same manner as in Example 6, the Sc23DOX gene was linked to pBin+201 to prepare a vector pBin+201_Sc23DOX. This was introduced to *Agrobacterium Rhizogenes* C15834 strain. The vector-containing *Agrobacterium* was cultured under shaking at 28° C. for 12 hours in YEB liquid culture medium [5 g/L beef extract, 1 g/L yeast extract, 5 g/L peptone, 5 g/L sucrose, 2 mM magnesium sulfate (pH 7.2)] containing 50 ppm kanamycin. The culture solution was spread on YEB agar culture medium (2% agarose), and was cultured at 28° C. for 72 hours in a dark place. The potato variety "Konafubuki" cultured in vitro was cut into 1 to 1.5 cm pieces, and the tip of the root side of the stem was attached to the colony of *Rhizogenes*, and was stuck to B5 culture medium (containing 0.3% Gelrite and 2% sucrose) in a plant box so that the tip of the root side faced upward. This was cultured at 20° C. for 20 days in a dark place. The upper part of the stem in which formation of hairy roots had been confirmed was cut and was transferred to MS culture medium (containing 0.3% Gelrite, 2% sucrose, and 250 ppm Cefotaxime). The upper part of the stem was cultured at 25° C. for 7 days in a dark place and was sterilized. The tip (1 cm) of the grown hairy roots was cut, was transferred to B5 culture medium (containing 0.3% Gelrite, 2% sucrose, and 250 ppm Cefotaxime), and was cultured at 25° C. for 7 days in a dark place. The obtained hairy roots were cut into slices, the slices were transferred to B5 liquid culture medium (containing 2% sucrose), and were further cultured under shaking (100 rpm) at 20° C. for 14 days in a dark place. Then, 100 mg of products that proliferated from the slices were frozen with liquid nitrogen, and were crushed with a mixer mill (1/30 sec, 2 min). Then, 300 µL of methanol was added to the crushed products and the mixture was sonicated for 10 minutes. The mixture was subjected to centrifugal separation (15,000 rpm, 10 min), and the supernatant was collected. This extraction operation was repeated three times, the collected supernatant was dried under reduced pressure, and the residues were dissolved again in 200 µL of methanol. Then, 20 µL of the re-dissolved solution was dissolved in 180 µL of methanol, and glycoalkaloid was analyzed through LC-MS (manufactured by Waters, product name: UPLC-ESI-MS ACQUITY). As the column, ACQUITY HSS T3 (1.8 µm φ2.1×100 mm) (manufactured by Waters) was used. In LC analysis, gradient elution was performed using a mobile phase (A: 0.1% formic acid water and B: acetonitrile). The gradient elution was performed under the condition: 0 to 30 minutes, 90% A/10% B to 45.00 A/55.0% B; 30 to 31 minutes, 45.0% A/55.0% B to 0% A/100% B; and maintaining for from 31 to 35 minutes, 100% B. As a result, as shown in FIGS. 14 and 15, it was confirmed that leptine I and leptine II can be produced by compulsively expressing only the Sc23DOX gene in Konafubuki that had not been recognized to produce leptine.

As described above, it was suggested that lines and varieties having the Sc23ACT gene can produce leptine by introducing only the Sc23DOX gene.

5 (Example 15) Acquisition of Gene Sequences of Promotor of 23ACT Gene Having Functions and Promotor of 23ACT Gene Having No Function

SRX118622: transcriptome analysis of breaker fruit of 10 *Solanum lycopersicum* cv Heinz registered in NCBI data base was used to analyze expression of the S123ACT gene, and Trinity (<https://github.com/trinityrnaseq/trinityrnaseq/wiki>), Bowtie (<http://bowtie-bio.sourceforge.net/index.shtml>), DEGseq (Original site), and express (Original site) were used to analyze the gene expression level (fpkm: fragments per kilobase of exon per million reads mapped). As a result, the calculated gene expression level was found to be 70.93. Because esculeoside A is synthesized in the breaker fruit stage of tomato, a sufficient level of the gene 15 23ACT is found to be expressed. *Solanum chacoense* M6 (Leisner et al., Plant Journal (2018) 94, 562-570) is the line that produces no leptine. However, as a result of homology search of the published gene information, the 23ACT gene was found to be g38106. Moreover, it was found that the 20 promoter sequence of Sc23ACT in *Solanum chacoense* M6 can function because the described gene expression level in a leaf was 63.11. The genome sequence on which g38106 was located was found to be scaffold_1344 from Table S5, and an effective sequence (SEQ ID NO: 34) as a promoter 25 30 was obtained. Based on this sequence and the sequences of g38106 and St23ACT, using primers U1240 (TCAGCAATAGTGCATTACCAAGAG) (SEQ ID NO: 35) and U1241 (CGCCTAACGTGAAGAAGGGGTA) (SEQ ID NO: 36), one sequence (SEQ ID NO: 37) was obtained from 35 40 50 *S. chacoense* PI 458310, two sequences (A: SEQ ID NO: 38 and B: SEQ ID NO: 39) were obtained from Konafubuki, and one sequence (SEQ ID NO: 40) was obtained from Sassy. The sequence B of Konafubuki and the sequence of Sassy are completely identical to each other, and are found to have no activity. On the other hand, *Solanum chacoense* M6 and the sequence A of Konafubuki have activity. The results obtained by comparing these sequences are shown in FIG. 16A to FIG. 16C. By comparing these sequences, it is possible to change a generally inactive type to the St23ACT active type by substituting an inactive sequence with an active sequence through a method such as gene recombination, genome editing, or the like.

50 (Example 16) Acquisition of Gene Sequences of Promotor of 23DOX Gene Having Functions and Promotor of 23DOX Gene Having No Function

SRX118622: transcriptome analysis of breaker fruit of 55 *Solanum lycopersicum* cv Heinz registered in NCBI data base was used to analyze expression of the S123DOX gene, and Trinity (<https://github.com/trinityrnaseq/trinityrnaseq/wiki>), Bowtie (<http://bowtie-bio.sourceforge.net/index.shtml>), DEGseq (Original site), and express (Original site) were used to analyze the gene expression level (fpkm: fragments per kilobase of exon per million reads mapped). As a result, the calculated gene expression level was found to be 1982.30. Because esculeoside A is synthesized in the breaker fruit stage of tomato, a sufficient amount of the gene 60 65 70 75 80 85 90 95 23DOX is found to be expressed. *Solanum chacoense* M6 (Leisner et al., Plant Journal (2018) 94, 562-570) is a line that produces no leptine. However, as a result of homology search of the published gene information, the 23DOX gene

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was found to be g39095. Moreover, it was found that the promoter sequence of Sc23DOX in *Solanum chacoense* M6 does not function because the described gene expression level in a leaf was 2.25. The genome sequence on which g39095 was located was found to be scaffold 1570 from Table S5, and the sequence (SEQ ID NO: 41) that does not function as a promoter was obtained. Based on this sequence and the sequences of g39095 and St23DOX, using primers U1249 (GGGTCCGACTTTTGTGTTT) (SEQ ID NO: 42) and U1243 (CAATGGCAATTGTGGAATCA) (SEQ ID NO: 43), two functional sequences (A: SEQ ID NO: 44 and B: SEQ ID NO: 45) were obtained from *S. chacoense* PI 458310. However, since no functional sequence was obtained from Sassy or Konafubuki, primers U1276 (TAAAATTATTCAATTTCATAAAATTGACA) (SEQ ID NO: 46) and U1243 were used to obtain two sequences (A: SEQ ID NO: 47 and B: SEQ ID NO: 48) from Sassy, and the sequence (SEQ ID NO: 49) from Konafubuki, which is identical to the sequence A of Sassy and has no activity. The results obtained by comparing these sequences are shown in FIG. 17A to FIG. 17E. By comparing these sequences, it is

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possible to change a generally inactive type to the St23DOX active type by substituting an inactive sequence with an active sequence through a method such as gene recombination, genome editing, or the like.

INDUSTRIAL APPLICABILITY

As described above, according to the present invention, use of the identified hydroxylase gene and/or acetyltransferase gene can introduce a hydroxy group or an acetoxy group into position 23 of a spirosolane skeleton, and can produce leptinine or leptine. Moreover, according to the present invention, biosynthesis and accumulation of leptine can also increase, for example, resistance against CPB in plants. That is, plants having increased resistance against CPB can be efficiently provided. According to the present invention, it is possible to efficiently determine, for example, resistance against CPB in plants by using an indicator, for example, the presence of the gene. Therefore, the present invention is effective in cultivating, for example, solanaceous plants that experience insect damage by CPB.

SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 49

<210> SEQ ID NO 1
<211> LENGTH: 960
<212> TYPE: DNA
<213> ORGANISM: Solanum chacoense

<400> SEQUENCE: 1

<210> SEQ ID NO 2
<211> LENGTH: 320
<212> TYPE: PRT
<213> ORGANISM: *Solanum chacoense*

<400> SEQUENCE: 2

Met	Ala	Ser	Thr	Lys	Ser	Val	Lys	Val	Pro	Thr	Ile	Asp	Phe	Ser	Asn
1				5					10						15

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-continued

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

<210> SEQ ID NO 3
 <211> LENGTH: 960
 <212> TYPE: DNA
 <213> ORGANISM: Solanum tuberosum

<400> SEQUENCE: 3

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atggcatcta ccaaatcagt taaaatcccc gccattgatt tttccaaatta tcaagagcta 60
aaaccaaaca ctccattatg ggaatccaca aaaattcaaa ttttgaagc tttacaagaa 120
tatggttgtt ttgaagcaat atataaagtt ccaaatgaaa ttaaagatgg aatgttttgtt 180
attcctaaag aaatatttga atttccttta gaaaccaaatt tgaaaaattt ctcagaaaaa 240
ccattacatg gctacatggg aatgattcca caattgccc tttatgttgc 300
cctgattgc ttaatcgta aagtcttcaa acttttctt atatctttgc gcctcatgg 360
aatcaacatt tctgcgattt ggttttttttctt tattctaattt cacttgcgttgc attggatgag 420

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atgttggaaa ggatgattc ggagaatttg ggattaaaaa atcacattga tgaatttttg	480
aataccaaatt atttccttatt tagatttaca cattataagg gatcatcaat tattagtggaa	540
gatgaaaata ataaaactac tggattgggt ggccacacag atggtaactt cttaactttt	600
atatcacaaa atcaagtcaa tggattgcaa atcaacaaaatggagagtg gattgtatgt	660
aatatttcac caaattcttg tggatgtttg gctggtgatt cttcaaaagc atggacaaat	720
ggtcaattac atttcctct ccacagagta acaattgccc gagaaagtga tagactttct	780
attcaattat ttcatttatac aaaaccaggta cacttcattcg aggccccaca agaactggtg	840
gatgaagaac acccttact cttcaagcca ttggaaattc ttggattatt ttggatgtct	900
gcctcagaag ctggctatgg agctcctccc agtgatctt tcaagactta ttgcgggttt	960

<210> SEQ ID NO 4

<211> LENGTH: 320

<212> TYPE: PRT

<213> ORGANISM: Solanum tuberosum

<400> SEQUENCE: 4

Met Ala Ser Thr Lys Ser Val Lys Val Pro Ala Ile Asp Phe Ser Asn	
1 5 10 15	

Tyr Gln Glu Leu Lys Pro Asn Thr Pro Leu Trp Glu Ser Thr Lys Ile	
20 25 30	

Gln Ile Phe Glu Ala Leu Gln Glu Tyr Gly Cys Phe Glu Ala Ile Tyr	
35 40 45	

Lys Val Pro Asn Glu Ile Lys Asp Gly Met Phe Gly Ile Ser Lys Glu	
50 55 60	

Ile Phe Glu Phe Pro Leu Glu Thr Lys Leu Lys Asn Phe Ser Glu Lys	
65 70 75 80	

Pro Leu His Gly Tyr Met Gly Met Ile Pro Gln Leu Pro Leu Tyr Glu	
85 90 95	

Ser Leu Cys Ile Pro Asp Leu Leu Asn Arg Gln Ser Leu Glu Thr Phe	
100 105 110	

Ser Asn Ile Phe Trp Pro His Gly Asn Gln His Phe Cys Asp Leu Val	
115 120 125	

Lys Ser Tyr Ser Asn Pro Leu Val Glu Leu Asp Glu Met Leu Lys Arg	
130 135 140	

Met Ile Ser Glu Asn Leu Gly Leu Lys Asn His Ile Asp Glu Leu Leu	
145 150 155 160	

Asn Thr Asn Tyr Phe Leu Phe Arg Phe Thr His Tyr Lys Gly Ser Ser	
165 170 175	

Ile Ile Ser Gly Asp Glu Asn Asn Lys Thr Thr Gly Leu Gly His	
180 185 190	

Thr Asp Gly Asn Phe Leu Thr Phe Ile Ser Gln Asn Gln Val Asn Gly	
195 200 205	

Leu Gln Ile Asn Lys Asn Gly Glu Trp Ile Asp Val Asn Ile Ser Pro	
210 215 220	

Asn Ser Cys Val Val Leu Ala Gly Asp Ser Phe Lys Ala Trp Thr Asn	
225 230 235 240	

Gly Gln Leu His Ser Pro Leu His Arg Val Thr Ile Ala Gly Glu Ser	
245 250 255	

Asp Arg Leu Ser Ile Gln Leu Phe Ser Leu Ser Lys Pro Gly His Phe	
260 265 270	

Ile Glu Ala Pro Gln Glu Leu Val Asp Glu Glu His Pro Leu Phe	
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45**46**

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275 280 285

Lys Pro Phe Glu Ile Leu Gly Leu Phe Gly Tyr Ala Ala Ser Glu Ala
 290 295 300

Gly Tyr Gly Ala Pro Pro Ser Asp Leu Phe Lys Thr Tyr Cys Gly Val
 305 310 315 320

<210> SEQ ID NO 5
<211> LENGTH: 963
<212> TYPE: DNA
<213> ORGANISM: Solanum lycopersicum

<400> SEQUENCE: 5

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aaacccaaca ctccactatg	ggaatccaca	aaaattcaag	tttttgaagc	ttttcaagaa	120
tatggttgtt ttgaagcaat	atatgataaa	gttccaaat	aaatttagaga	ggaaacattt	180
gatatgtcaa aagaaatatt	tgaatttcctt	ttagatacta	aagtaaaaaa	tatccagaa	240
aaaccaatgc atggatata	ggaatgattt	ccacaattgc	cattgtatga	gagtttgtt	300
attcctgatt tgcttaatcc	tcaaagtctt	caaaattttt	ctaataatctt	ttggcctcag	360
ggtaatcaac atttctgcaa	tttggtaaag	tcttattcta	atccacttgt	ggaattggat	420
gagattttga aaaggatgat	ttcggagaat	tttgagattaa	aaattcacat	tgtatgattt	480
ttgaatgccca attatttcctt	attnagattt	acacattaca	agggatcatc	aattgctagt	540
ggagatgaaa ataataaaagc	tgctggattt	ggtggccaca	cggatggtaa	cttcttgact	600
tttatatcgc aaaatcaagt	taatggattt	caaataaca	aaaatggaga	atggattgat	660
gtgattattt caccaatttc	ttacgttgtt	ttggccggtg	attccttcaa	agcttggaca	720
aatggtcgat tgcattcacc	tctccacaga	gtaacaatgt	ccggacaaaa	tgatagactc	780
tccattcaat tgtttcatt	atcaaagccca	ggtcacttca	tccaggcacc	aaaagaacta	840
gtagatgaag aacacccatt	actcttcaag	ccatttgaatt	ttcttgaattt	attcaagtat	900
ggtaccacag aagctggcta	tacagctcctt	ccaagtgatc	ttttcaagat	ttatttggtt	960
gtt					963

<210> SEQ ID NO 6
<211> LENGTH: 321
<212> TYPE: PRT
<213> ORGANISM: Solanum lycopersicum

<400> SEQUENCE: 6

Met Ala Ser Ile Lys Ser Val Lys Val Pro Thr Ile Asp Phe Ser Asn	5	10	15
---	---	----	----

Tyr Gln Glu Leu Lys Pro Asn Thr Pro Leu Trp Glu Ser Thr Lys Ile	20	25	30
---	----	----	----

Gln Val Phe Glu Ala Phe Gln Glu Tyr Gly Cys Phe Glu Ala Ile Tyr	35	40	45
---	----	----	----

Asp Lys Val Pro Asn Glu Ile Arg Glu Glu Thr Phe Asp Met Ser Lys	50	55	60
---	----	----	----

Glu Ile Phe Glu Phe Pro Leu Asp Thr Lys Val Lys Asn Ile Ser Glu	65	70	75	80
---	----	----	----	----

Lys Pro Met His Gly Tyr Met Gly Met Ile Pro Gln Leu Pro Leu Tyr	85	90	95
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Glu Ser Leu Cys Ile Pro Asp Leu Leu Asn Pro Gln Ser Leu Gln Asn	100	105	110
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Phe Ala Asn Ile Phe Trp Pro Gln Gly Asn Gln His Phe Cys Asn Leu
115 120 125

Val Lys Ser Tyr Ser Asn Pro Leu Val Glu Leu Asp Glu Ile Leu Lys
130 135 140

Arg Met Ile Ser Glu Asn Leu Arg Leu Lys Ile His Ile Asp Glu Leu
145 150 155 160

Leu Asn Ala Asn Tyr Phe Leu Phe Arg Phe Thr His Tyr Lys Gly Ser
165 170 175

Ser Ile Ala Ser Gly Asp Glu Asn Asn Lys Ala Ala Gly Leu Gly Gly
180 185 190

His Thr Asp Gly Asn Phe Leu Thr Phe Ile Ser Gln Asn Gln Val Asn
195 200 205

Gly Leu Gln Ile Asn Lys Asn Gly Glu Trp Ile Asp Val Ile Ile Ser
210 215 220

Pro Asn Ser Tyr Val Val Leu Ala Gly Asp Ser Phe Lys Ala Trp Thr
225 230 235 240

Asn Gly Arg Leu His Ser Pro Leu His Arg Val Thr Met Ser Gly Gln
245 250 255

Asn Asp Arg Leu Ser Ile Gln Leu Phe Ser Leu Ser Lys Pro Gly His
260 265 270

Phe Ile Gln Ala Pro Lys Glu Leu Val Asp Glu Glu His Pro Leu Leu
275 280 285

Phe Lys Pro Phe Glu Ile Leu Glu Leu Phe Lys Tyr Gly Thr Thr Glu
290 295 300

Ala Gly Tyr Thr Ala Pro Pro Ser Asp Leu Phe Lys Ile Tyr Cys Gly
305 310 315 320

Val

<210> SEQ ID NO 7
<211> LENGTH: 1302
<212> TYPE: DNA
<213> ORGANISM: Solanum chacoense

<400> SEQUENCE: 7

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ccttcttcac ttaggegtta caatctttgt ctaacagatc aaatcatgg tccagtctac      120
atgccaatttgc tagcctttta tcctttctcc tctaaaacac cacaacaat atctaaccatt      180
cttggaaattt cactatccaa agtttgc tcttattatc catttgctgg aacactcgaa      240
gatgacaaca cttttgtcga ttgcaacat aggggagcca aatttatgaa cgttcgatac      300
gattgtccat tgtctgaaat cgtaatctt ccagacactg gccctgaata tctaccttt      360
gctaaaggta tgccttgggg ttcaactcca gatgacaaaa gtttacttgt tgtccaatta      420
agccatttta attgcggagg attagctata agtgttaggc tatcacacaa aattgctgat      480
ggatgcacac tctgcaattt tattagtat tgggcttcca tagctcgtga tgagaacgcg      540
aatatacctt cccctgaaat gattggatcg tccattttc cgccatccac tgaaatgcca      600
tccactggca ttcacatggta tactgagttt gattatgaat tttacaattt gcccgttatt      660
aaaaaaaaaggta acctattttca caattcgaaa cttagatgc tgaaaagtca agtgcacatca      720
gaaacagggg tgcaaatcc tagccgactt gaagtgtgt ttgcactaat ttacaagtgt      780
gctgcaacag cagctcgagc aaactcgago tcgtttaaac gatcctcggt gtcactacct      840
gtaaatttac gtccaatattt ggatccacca ctagcaacac ggacaatagg gaatatttt      900

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49**50**

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agtttatca aagtggaaac aatgagttag gatgaaatga caatgcac agtggttcgt	960
gagataagga aaggtaaaga agaactgaag aaggaggggc atgtggagga gaagaagttt	1020
gtgtcgctt ggtctgagt gatatattcg atggtaaag aaattgaact gtatcgaagt	1080
agcagtgttt gcaattgccc attgaataat ttggattttt gatggggaaa gccaagcagg	1140
gtaacaattc cagtatatgg gactgcaaac acctgcattt ttatggataa tctaagtgg	1200
gatgggattt acgtaattat tgtattacca gaaaaagacg tgactcaatt tgagaatagc	1260
aaagacctca tccagttctc ttctccaatt actaatctta at	1302

<210> SEQ ID NO 8

<211> LENGTH: 434

<212> TYPE: PRT

<213> ORGANISM: Solanum chacoense

<400> SEQUENCE: 8

Met Ala Ala Ser Ser Cys Val Ser Leu Val Glu Lys Phe Ile Lys Pro	
1 5 10 15	

Asp Ser Pro Thr Pro Ser Ser Leu Arg Arg Tyr Asn Leu Cys Leu Thr	
20 25 30	

Asp Gln Ile Met Val Pro Val Tyr Met Pro Ile Val Ala Phe Tyr Pro	
35 40 45	

Phe Ser Ser Lys Thr Pro Gln Gln Ile Ser Asn Ile Leu Glu Asn Ser	
50 55 60	

Leu Ser Lys Val Leu Ser Ser Tyr Tyr Pro Phe Ala Gly Thr Leu Gly	
65 70 75 80	

Asp Asp Asn Thr Phe Val Asp Cys Asn Asp Arg Gly Ala Lys Phe Met	
85 90 95	

Asn Val Arg Tyr Asp Cys Pro Leu Ser Glu Ile Val Asn Leu Pro Asp	
100 105 110	

Thr Gly Pro Glu Tyr Leu Pro Phe Ala Lys Gly Met Pro Trp Gly Ser	
115 120 125	

Thr Pro Asp Asp Lys Ser Leu Leu Val Val Gln Leu Ser His Phe Asn	
130 135 140	

Cys Gly Gly Leu Ala Ile Ser Val Arg Leu Ser His Lys Ile Ala Asp	
145 150 155 160	

Gly Cys Thr Leu Cys Asn Phe Ile Ser Asp Trp Ala Ser Ile Ala Arg	
165 170 175	

Asp Glu Asn Ala Asn Ile Pro Ser Pro Glu Met Ile Gly Ser Ser Ile	
180 185 190	

Phe Pro Pro Ser Thr Glu Met Pro Ser Thr Gly Ile His Met Asp Thr	
195 200 205	

Glu Phe Asp Tyr Glu Phe Tyr Asn Leu Pro Val Ile Lys Lys Arg Tyr	
210 215 220	

Leu Phe Ser Asn Ser Lys Leu Glu Met Leu Lys Ser Gln Val Thr Ser	
225 230 235 240	

Glu Thr Gly Val Gln Asn Pro Ser Arg Leu Glu Val Leu Phe Ala Leu	
245 250 255	

Ile Tyr Lys Cys Ala Ala Thr Ala Ala Arg Ala Asn Ser Ser Ser Phe	
260 265 270	

Lys Arg Ser Ser Leu Ser Leu Pro Val Asn Leu Arg Pro Ile Leu Asp	
275 280 285	

Pro Pro Leu Ala Thr Arg Thr Ile Gly Asn Ile Leu Ser Phe Ile Lys	
290 295 300	

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Val Glu Thr Met Ser Glu Asp Glu Met Thr Ile Ala Arg Val Val Arg
305 310 315 320

Glu Ile Arg Lys Gly Lys Glu Glu Leu Lys Lys Glu Gly His Val Glu
325 330 335

Glu Lys Lys Leu Val Ser Leu Trp Ser Glu Trp Ile Tyr Ser Met Gly
340 345 350

Lys Glu Ile Glu Leu Tyr Arg Ser Ser Ser Val Cys Asn Cys Pro Leu
355 360 365

Asn Asn Leu Asp Phe Gly Trp Gly Lys Pro Ser Arg Val Thr Ile Pro
370 375 380

Val Tyr Gly Thr Ala Asn Thr Cys Met Phe Met Asp Asn Leu Ser Gly
385 390 395 400

Asp Gly Ile Asp Val Ile Ile Val Leu Pro Glu Lys Asp Val Thr Gln
405 410 415

Phe Glu Asn Ser Lys Asp Leu Ile Gln Phe Ser Ser Pro Ile Thr Asn
420 425 430

Leu Asn

<210> SEQ ID NO 9
<211> LENGTH: 1302
<212> TYPE: DNA
<213> ORGANISM: Solanum tuberosum

<400> SEQUENCE: 9

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ccttccttcac ttaggcgtta caatctttgt ctaattgatc aaatcatgg tccagtctac	120
atgccaattt tagcctttta tcctttctcc tctaaaacac cacaacaagt atccagcatt	180
cttgaaaatt cactatccaa agtttgcgtcc tcttattatc catttgcgtgg aacactcgga	240
gataacaaca cttttgtcga ttgcaacat aggggagcca aatttatgaa cgttcgatac	300
gattgtccaa tgtctgaaat cgtcaatctt ccggacactg gcccgtataa tctaccttt	360
gctaaaggta tgccctgggg ttcaactcca gatgacaaaa gtttacttgt tggcaattt	420
agccattttt attgcggagg attagctata agtgttaggc tatcacacaa aattgctgat	480
ggatgcacac tctgcaattt tattagtat tggcgttcca tagctgtga tgagaacgcg	540
aatatacctt cccctgaaat gattggatcg tccattttc tgccatccac tgaaatgcca	600
tccactggca ttcacatgga tactgagggtt gattatgaaat tttacaattt acccgtagt	660
aaaaaaaaaggc acctattttc caattcaaaa cttgagatgc tgaaaagtca agtggcatca	720
gaaacagggg tgcaagatcc tagccgagtt gaagtgttgtt ttgcactaat ttacaagtgt	780
gctgcaacag cagttcgagc aaacttgagc ttgtttaaac gatcttcatt gtcaataacct	840
gtgaacttac gtccaatatt ggatccacca ctagcaacac ggacaatagg gaatattctt	900
agttttatca aagtggaaac aatgagtgag gatgaaatgca caattggcag aatggttcgc	960
gagataagaa aagctaaaga tgaagtgagg aaggaggggc atgtgaaggaa ggagaagcta	1020
gtgtcgcttt ggactgagtg gatatattcg atggtaaaat aaattgaaatt ttatagaagt	1080
agcagtggtt gcaattaccc attgaataat ttggattttg gattggaaa gccaaaggcagg	1140
gtaacaattt cagttatatgg gattgccaac acctgcattt ttatggataa tctaagtggaa	1200
gatgggattt aggtacttat tgcattacca gaaaaagacg tgactcaatt tgagaataac	1260
aaaagagctcc tccagttgg ttctccaatt actaatctta at	1302

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<210> SEQ ID NO 10
<211> LENGTH: 434
<212> TYPE: PRT
<213> ORGANISM: Solanum tuberosum

<400> SEQUENCE: 10

Met Ala Ala Ser Ser Cys Val Ser Leu Ala Glu Lys Ile Ile Lys Pro
1 5 10 15

Asp Ser Ser Thr Pro Ser Ser Leu Arg Arg Tyr Asn Leu Cys Leu Ile
20 25 30

Asp Gln Ile Met Val Pro Val Tyr Met Pro Ile Val Ala Phe Tyr Pro
35 40 45

Phe Ser Ser Lys Thr Pro Gln Gln Val Ser Ser Ile Leu Glu Asn Ser
50 55 60

Leu Ser Lys Val Leu Ser Ser Tyr Tyr Pro Phe Ala Gly Thr Leu Gly
65 70 75 80

Asp Asn Asn Thr Phe Val Asp Cys Asn Asp Arg Gly Ala Lys Phe Met
85 90 95

Asn Val Arg Tyr Asp Cys Pro Met Ser Glu Ile Val Asn Leu Pro Asp
100 105 110

Thr Gly Pro Glu Tyr Leu Pro Phe Ala Lys Gly Met Pro Trp Gly Ser
115 120 125

Thr Pro Asp Asp Lys Ser Leu Leu Val Val Gln Leu Ser His Phe Asn
130 135 140

Cys Gly Gly Leu Ala Ile Ser Val Arg Leu Ser His Lys Ile Ala Asp
145 150 155 160

Gly Cys Thr Leu Cys Asn Phe Ile Ser Asp Trp Ala Ser Ile Ala Arg
165 170 175

Asp Glu Asn Ala Asn Ile Pro Ser Pro Glu Met Ile Gly Ser Ser Ile
180 185 190

Phe Leu Pro Ser Thr Glu Met Pro Ser Thr Gly Ile His Met Asp Thr
195 200 205

Glu Val Asp Tyr Glu Phe Tyr Asn Leu Pro Val Ser Lys Lys Arg Tyr
210 215 220

Leu Phe Ser Asn Ser Lys Leu Glu Met Leu Lys Ser Gln Val Ala Ser
225 230 235 240

Glu Thr Gly Val Gln Asp Pro Ser Arg Val Glu Val Leu Phe Ala Leu
245 250 255

Ile Tyr Lys Cys Ala Ala Thr Ala Val Arg Ala Asn Leu Ser Leu Phe
260 265 270

Lys Arg Ser Ser Leu Ser Ile Pro Val Asn Leu Arg Pro Ile Leu Asp
275 280 285

Pro Pro Leu Ala Thr Arg Thr Ile Gly Asn Ile Leu Ser Phe Ile Lys
290 295 300

Val Glu Thr Met Ser Glu Asp Glu Met Thr Ile Gly Arg Met Val Arg
305 310 315 320

Glu Ile Arg Lys Ala Lys Asp Glu Val Arg Lys Glu Gly His Val Lys
325 330 335

Glu Glu Lys Leu Val Ser Leu Trp Thr Glu Trp Ile Tyr Ser Met Gly
340 345 350

Lys Glu Ile Glu Phe Tyr Arg Ser Ser Ser Val Cys Asn Tyr Pro Leu
355 360 365

Asn Asn Leu Asp Phe Gly Leu Gly Lys Pro Ser Arg Val Thr Ile Pro
370 375 380

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Val	Tyr	Gly	Ile	Ala	Asn	Thr	Cys	Met	Phe	Met	Asp	Asn	Leu	Ser	Gly
385				390			395			400					

Asp	Gly	Ile	Glu	Val	Leu	Ile	Ala	Leu	Pro	Glu	Lys	Asp	Val	Thr	Gln
				405			410				415				

Phe	Glu	Asn	Asn	Lys	Glu	Leu	Leu	Gln	Phe	Gly	Ser	Pro	Ile	Thr	Asn
				420			425				430				

Leu Asn

<210> SEQ ID NO 11

<211> LENGTH: 1272

<212> TYPE: DNA

<213> ORGANISM: Solanum lycopersicum

<400> SEQUENCE: 11

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atgccaattt	tagccttttta	tccttaacccc	tctaaaacac	cagaacaagt	atccaacata	180
cttgaagatt	cactatccaa	agttttatcc	tcttactatc	catttgctgg	aacactcgga	240
ttagataacg	ctacttttgt	cgattgcaat	gacagggggag	ctaaatctat	acaggttcga	300
taacgattgtc	caatgtctga	aatagtcaat	cttccggata	ctggccctga	atatctacct	360
tttgctaaag	gtacgcctt	gagttcaact	ccagaggaaac	aaagtttact	agttgttcaa	420
ttaaggcatt	ttaattgcgg	aggatttagt	ataagtgcata	ggctatccca	taaaattgct	480
gatggatgca	cgctcgccaa	tttcattgt	gattggctt	ccgtagctcg	tgtgacaac	540
gcgaatatac	tttccccctca	attgatttgg	tcgtccattt	ttccgcattt	cactgaaatg	600
cgcattcaca	cggatactaa	cgttattat	gagtttaca	atctaccctg	ttgtaaaaaa	660
aggtaacttgc	tttccaacgc	gaaactttag	atgctgaaaa	cccaagtgg	atcagaaaca	720
gggggtgaaa	atccaaactcg	aatttgaatg	ctgtccgcac	taatttacaa	gtgtctgt	780
acagcaaaact	cgagctcg	tagaccatcc	tcgttgcac	tgccgggtgaa	tttaacttccg	840
atactgaatc	caccgtttaga	aacacggaca	gttagggata	ttttagttt	tatcaaagt	900
gaaacaacga	gtgaggatga	aatgacaatc	gggagagttgg	ttcgcgagat	taggaaaggt	960
aaagacgaat	tgaagcagga	aggggggtgt	aagaaggaga	agcttagttc	gctatggagt	1020
gagttggatac	attcgattga	cttgcacaga	agtagcgtt	tttgcattt	cccattgaat	1080
aatttggatt	ttggatgggg	aaaaccaaac	aggtagcaa	ttccctgtt	tggagttgca	1140
aacacctgca	tgttatgga	taatctaagt	ggagatggaa	ttgaggtt	tattgcatta	1200
ccagaaaaag	atgcactca	atttgagaat	agcaaagagc	ttctccactt	tgcttctcca	1260
gttacgaatc	tc					1272

<210> SEQ ID NO 12

<211> LENGTH: 424

<212> TYPE: PRT

<213> ORGANISM: Solanum lycopersicum

<400> SEQUENCE: 12

Met	Thr	Ala	Ser	Ser	Phe	Val	Ser	Met	Ala	Glu	Lys	Ile	Ile	Lys	Pro
1					5			10			15				

His	Ser	Pro	Thr	Pro	Phe	Ser	Val	Lys	Arg	Tyr	Asn	Leu	Cys	Leu	Met
					20			25			30				

Asp	Glu	Ile	Met	Val	Pro	Val	Tyr	Met	Pro	Ile	Val	Ala	Phe	Tyr	Pro
					35			40			45				

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Asn	Pro	Ser	Lys	Thr	Pro	Glu	Gln	Val	Ser	Asn	Ile	Leu	Glu	Asp	Ser
50															60
Leu	Ser	Lys	Val	Leu	Ser	Ser	Tyr	Tyr	Pro	Phe	Ala	Gly	Thr	Leu	Gly
65															80
Ser	Asp	Asn	Ala	Thr	Phe	Val	Asp	Cys	Asn	Asp	Arg	Gly	Ala	Lys	Ser
85															95
Ile	Gln	Val	Arg	Tyr	Asp	Cys	Pro	Met	Ser	Glu	Ile	Val	Asn	Leu	Pro
100															110
Asp	Thr	Gly	Pro	Glu	Tyr	Leu	Pro	Phe	Ala	Lys	Gly	Thr	Pro	Trp	Ser
115															125
Ser	Thr	Pro	Glu	Glu	Gln	Ser	Leu	Leu	Val	Val	Gln	Leu	Ser	His	Phe
130															140
Asn	Cys	Gly	Gly	Leu	Gly	Ile	Ser	Ala	Arg	Leu	Ser	His	Lys	Ile	Ala
145															160
Asp	Gly	Cys	Thr	Leu	Ala	Asn	Phe	Ile	Ser	Asp	Trp	Ala	Ser	Val	Ala
165															175
Arg	Asp	Asp	Asn	Ala	Asn	Ile	Pro	Ser	Pro	Gln	Leu	Ile	Gly	Ser	Ser
180															190
Ile	Phe	Pro	Pro	Phe	Thr	Glu	Met	Arg	Ile	His	Thr	Asp	Thr	Asn	Val
195															205
Asp	Tyr	Glu	Phe	Tyr	Asn	Leu	Pro	Val	Cys	Lys	Lys	Arg	Tyr	Leu	Phe
210															220
Ser	Asn	Ala	Lys	Leu	Glu	Met	Leu	Lys	Thr	Gln	Val	Glu	Ser	Glu	Thr
225															240
Gly	Val	Gln	Asn	Pro	Thr	Arg	Ile	Glu	Val	Leu	Ser	Ala	Ile	Tyr	
245															255
Lys	Cys	Ala	Val	Thr	Ala	Asn	Ser	Ser	Ser	Phe	Arg	Pro	Ser	Ser	Leu
260															270
Ser	Leu	Pro	Val	Asn	Leu	Arg	Pro	Ile	Leu	Asn	Pro	Pro	Leu	Glu	Thr
275															285
Arg	Thr	Val	Gly	Asn	Ile	Ile	Ser	Phe	Ile	Lys	Val	Glu	Thr	Thr	Ser
290															300
Glu	Asp	Glu	Met	Thr	Ile	Gly	Arg	Val	Val	Arg	Glu	Ile	Arg	Lys	Gly
305															320
Lys	Asp	Glu	Leu	Lys	Gln	Glu	Gly	Val	Lys	Lys	Glu	Lys	Lys	Leu	Val
325															335
Ser	Leu	Trp	Ser	Glu	Trp	Ile	His	Ser	Ile	Asp	Leu	Tyr	Arg	Ser	Ser
340															350
Ser	Val	Cys	Asn	Tyr	Pro	Leu	Asn	Asn	Leu	Asp	Phe	Gly	Trp	Gly	Lys
355															365
Pro	Asn	Arg	Val	Ala	Ile	Pro	Val	Phe	Gly	Val	Ala	Asn	Thr	Cys	Met
370															380
Phe	Met	Asp	Asn	Leu	Ser	Gly	Asp	Gly	Ile	Glu	Val	Ile	Ile	Ala	Leu
385															400
Pro	Glu	Lys	Asp	Ala	Thr	Gln	Phe	Glu	Asn	Ser	Lys	Glu	Leu	Leu	His
405															415
Phe	Ala	Ser	Pro	Val	Thr	Asn	Leu								
420															

<210> SEQ ID NO 13
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:

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<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 13

ggatccatgg catcttatcaa atcag

25

<210> SEQ ID NO 14

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 14

ctcgagtc aaataccacaa taaatcttg

29

<210> SEQ ID NO 15

<211> LENGTH: 28

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 15

ctwaaaccaa acactycayw atggaaat

28

<210> SEQ ID NO 16

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 16

gggtgattywt catcyacwar ttcttttgg

29

<210> SEQ ID NO 17

<211> LENGTH: 25

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 17

tggtgattac cctgaggcca aaaga

25

<210> SEQ ID NO 18

<211> LENGTH: 24

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 18

ggtcgattgc attctcctgt ccac

24

<210> SEQ ID NO 19

<211> LENGTH: 29

<212> TYPE: DNA

<213> ORGANISM: Artificial Sequence

<220> FEATURE:

<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 19

catatggcat ctaccaaatac agttaaagt

29

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<210> SEQ ID NO 20
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence
<400> SEQUENCE: 20

gtcgactcaa acaccgcaat aagtcttga 29

<210> SEQ ID NO 21
<211> LENGTH: 36
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 21

ggatccata tgacagcatc aagtttgta tctatg 36

<210> SEQ ID NO 22
<211> LENGTH: 30
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 22

gtcgacccat agattcgtaa ctggagaago 30

<210> SEQ ID NO 23
<211> LENGTH: 24
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 23

tgcacatcac tggcattcac atgg 24

<210> SEQ ID NO 24
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 24

catatggcag catcaagttg tgtat 25

<210> SEQ ID NO 25
<211> LENGTH: 33
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 25

gtcgacttaa ttaagattag taattggaga aga 33

<210> SEQ ID NO 26
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

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<400> SEQUENCE: 26

ggcatctacc aaatcagttt aag

23

<210> SEQ ID NO 27
<211> LENGTH: 22
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 27

gtcttgaaaa catcaactggg ag

22

<210> SEQ ID NO 28
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 28

gattatgaat tttacaattt g

21

<210> SEQ ID NO 29
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 29

tacaggtagt gacaacgagg atc

23

<210> SEQ ID NO 30
<211> LENGTH: 29
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 30

caccatggca tctaccaaat cagttaaag

29

<210> SEQ ID NO 31
<211> LENGTH: 25
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 31

tcaaacacccg caataagtct tgaaa

25

<210> SEQ ID NO 32
<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 32

catatggcag catcaagttg tgt

23

<210> SEQ ID NO 33
<211> LENGTH: 32

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<212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 33

gtcgacttaa ttaagattag taattggaga ag 32

<210> SEQ ID NO 34
 <211> LENGTH: 1693
 <212> TYPE: DNA
 <213> ORGANISM: Solanum chacoense
 <220> FEATURE:
 <223> OTHER INFORMATION: 23ACT promoter

<400> SEQUENCE: 34

tca	gcaatag	tgcattacca	gagtaagacg	tgactcaatt	tgagaatagc	aaagagctcc	60
tcc	tgttcgc	ttctccaatt	acaataatca	agaatggaga	aatatagcaa	caatcgttt	120
gtt	gatttta	aagtgcgagt	gttacaatct	ctattattcc	tctaattcgc	ctttcaaat	180
ata	attcaag	gagtcttgag	cttgatctt	aatttgtcta	tgaacttggt	cttggattca	240
atg	actaatt	tagagttgt	tctagaatca	gaattaaatt	aattttttta	atagatatta	300
aaa	aatatttt	tctctttcta	aaataatctt	aattttaaaa	gtacattgtat	acttgcttt	360
ttt	cataattt	tgactctcaa	aagcaccttt	gacaaatatt	tgtcaaacac	aatttgctta	420
tga	aaaatcac	tttcaaaca	aatttagtcaa	acacaaattt	ttttttttt	caaagacagt	480
ttt	ttggaaa	gtatatttaa	aaaagtcctt	ttaaaaataa	gtaattttta	acagcaatgc	540
caa	aatagact	ctcataatct	aacaaaggta	tgagagtggg	ggcttgagga	tgtgatggc	600
aa	agtagtagg	cattgggggc	gttaggtggg	tagggatgtat	ataataaata	tacaatatta	660
ttt	atagaac	ttattttcc	aaccttttat	taaataagtc	attttctta	tttttaata	720
act	tatatttt	caaaagaata	attattttc	tccataccat	acgacaaatg	cacttaattt	780
tgc	atttattt	tgagattttt	ttcttcgaga	tttcctcta	aagtttttagc	tgaactagaa	840
cacac	ccat	tagttccctt	gttagttca	atccaaacaca	caccttagcc	caacactatt	900
tat	tttatca	actcataactt	atccccaca	cataactagg	gaaagegtat	gattaaacac	960
atattc	gatt	tggatgctgc	tatthaattt	gtattcattt	ttttaatata	tttttcacac	1020
gg	ttactcat	ttcaaataat	tttaggtata	tggtatttga	aatttagttgt	gacaaatcta	1080
actt	caaat	atatattaa	atttatatg	attgaagttt	gttattttt	atcaaactca	1140
agt	tattcta	tccaaagttc	atacacgtat	aactaacata	aaagcaatac	aaaataattt	1200
aa	actat	ttaatatta	attataaata	tatattttt	aattaatata	ttgtcaaact	1260
aatat	gtgt	gtttaggtt	tgaaacttac	actctcaata	gtttaggtat	gaaactcaaa	1320
aaa	aggggat	gttttaggtt	tgttttgac	acttatctt	attttaatgt	atgttttttta	1380
ctcaata	aca	atgtaaaaaa	acaattttt	gtttttttt	taatttgttg	tttaaaataaa	1440
ttatagata	aa	tttatggtt	ataaattattt	tcattaaaca	gttagcatct	atgctctcta	1500
aattt	gagca	tgtactagta	gacactaaa	cttgtttaaa	caatagacac	atatctacct	1560
agcataata	ac	cgatgtgatg	caacaacgta	ggcacaaat	gcacgttggc	tggatggcgt	1620
gctgggatgg	cg	gtgcctact	ataagtattt	aattttacca	tagttccca	tctacatcaa	1680
actcaaagga	aag						1693

<210> SEQ ID NO 35

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<211> LENGTH: 23
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 35

tcagcaatag tgcattacca gag

23

<210> SEQ ID NO 36
<211> LENGTH: 21
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 36

cgccctaagtg aagaagggt a

21

<210> SEQ ID NO 37
<211> LENGTH: 1693
<212> TYPE: DNA
<213> ORGANISM: S.chacoense
<220> FEATURE:
<223> OTHER INFORMATION: 23ACT promoter

<400> SEQUENCE: 37

tcagcaatag tgcattacca gagtaagacg tgactcaatt tgagaatagc aaagagctcc	60
tccgttgc ttctccaatt acaataatca agaatggaga aatatagcaa caatcgaaa	120
gttgattttt aagtgcgagt gttacaatct ctattattcc tctaattcgc cttttcaat	180
ataattcaag gagtcttgag cttgatctt aatttgtcta tgaacttgg tttggattca	240
atgactaatt tagagttgt tctagaatca gaattaaatt aattttttt atagatatta	300
aaaaatatttt tctctttcta aaataatctt aattttaaaa gtacattgtt acttgcttt	360
tttcataatt tgactctcaa aagcaccccc gacaaatattt tgtcaaacac aatttgctta	420
tgaaaatcac ttttcaaaaca aatttagtcaa acacaaatttgg tttttttttt caaagacagt	480
tttttggaaa gctattttaa aaaaggctt ttaaaaataa gtaatttttta acagcaatgc	540
caaataagact ctcataatct aacaaaggta tgagagtggg ggcttgagga tttgtatggc	600
aagtagtagg cattgggggc gtttaggtggg tagggaggat ataataataa tacaatattt	660
tttatagaac ttattttcc aacctttttaaataatctt attttctta ttttttaata	720
acttattttt caaaaagaata atttttttcc tccataccat acgacaaatgg cacttaattt	780
tgcattttt tgagattttt ttcttcgaga ttttcctcta aagtttttgc tgaacttagaa	840
cacacgcccattt taggttccctt gtttagttca atccaaacaca caccttagcc caacactatt	900
tatTTTATCA actcataactt attcccaaca cataactagg gaaagcgtat gattaaacac	960
atatttcgatt tggatgtgc tatTTTAAATT gtattcattt ttttaataata tttttcacac	1020
ggtttactcat ttcaaataat tttaggtata tggtatttgc aatttagttgtt gacaatcta	1080
acttcaaatg caatatttaa attttatatg attgaagttt gtttattttt atcaaactca	1140
agttattctt tccaaaggttc atacacgtat aactaacata aaagcaatac aaaaataattt	1200
aaactatTTTt ttaatatttaa attataaataa tatTTTattt aattaatata ttgtcaaaact	1260
aatatgtatg agtttaggtt tgaaaacttac actctcaata gtttaggtat gaaactcaaa	1320
aaaaggggat agtttaggtt tgTTTTGAC acttatctt atTTTAAATTt agtttttttta	1380
ctcaataacaa agtgtaaaaa acaattttttt gttttttttt taatttggat tttaaaataaa	1440

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ttagataat ttttatggtt ataaattatt tcattaaaca gtttagcatct atgtctctta	1500
aatttgagca tgtaactaga gacactaaa ctgtttaaa caatagacac atatctacct	1560
agcataatac acgtgtatg caacaacgta ggcacaaatt gcacgttgc tggatggcgt	1620
gctggatgg cgtgcctact ataagtattg aattttacca tagctccca tctacatcaa	1680
actcaaaqqq aaq	1693

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<210> SEQ ID NO 38
<211> LENGTH: 1689
<212> TYPE: DNA
<213> ORGANISM: S.tuberosum
<220> FEATURE:
<223> OTHER INFORMATION: 23ACT promoter A

<400> SEQUENCE: 38
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<210> SEQ ID NO 39
<211> LENGTH: 1676
<212> TYPE: DNA
<213> ORGANISM: *S.tuberosum*
<220> FEATURE:
<223> OTHER INFORMATION: 23ACT promoter B

<400> SEQUENCE: 39

ttagatcca	tgactcaatt	tgagaatgc	aaagagctcc	60		
tccgttcgc	ttctccaatt	acaataatca	agaatggaga	aatatagcaa	caatcgaaa	120
tttttttta	aagtgcgagt	gttacagtct	ctattattcc	tctgattcgt	cttttcaat	180
ataattcaag	gagtcggag	cttgatctt	aatttgtcta	tgaacttgg	cttggatca	240
atgactaatt	tagagttgt	tctagaatca	gaattaaatt	aattttttta	atagatatta	300
agaatatttt	tcttttcta	aaataatctt	aattttaaaa	gtacattgt	acttgcttt	360
tttcataatt	tgactctaa	aagcactttt	gacaaatatt	tgtcaaacac	aatttgctta	420
tggaaatcac	ttttcaaca	aattagtca	acacaaattt	tttttttttc	aaagacagtt	480
ttttggaaag	ctatttaaa	aaagtcctt	taaaaataag	taatttttaa	cagcaatgcc	540
aaatagactc	tcataatcta	acaaaaggat	gagagtgggg	gtttgaggat	gtgatggatca	600
atgtatggc	attggggcgc	ttaggtgggt	agggagtttata	caataaaat	acaatattat	660
ttatagaact	tattttcca	accttttatt	aaataagtca	tttttctt	tttttataaa	720
cctattttc	aaaaaaataa	ttattttct	ccataaccata	cgacaaatgc	acttaattgt	780
gcattttttt	gagatttttt	tctcgagat	tttcctctga	agtttttagct	gaactagaac	840
acacgccatt	aggttcctt	ttagttcaa	tccaaacacac	accttagccc	aacactattt	900
attttatcaa	ctcatactta	ttccaaacac	aaaacttaggg	aaagcgat	attaaacaca	960
tattcgattt	ggatgctgct	atthaattt	tattcattt	ttaatacat	tttcacacg	1020
gttactcatt	tcaaataatt	ttaggttat	gttatttga	attagttga	caaaatctaa	1080
cttcaaatgc	aatatttaaa	tttttatga	ttgaagttt	gttatttttta	tcaaactcaa	1140
gttattctat	ccaaagttca	tacacgtata	actaacataa	aagcaataca	aaaataattt	1200
aaactattttt	ttaatattaa	ttataaaat	atatttatta	attaatata	tgtcaaacta	1260
atatgtata	gttttaggttt	gaaacttaca	ctctcaatag	tttaggtatg	aaactcaaaa	1320
aaagaggata	gttttaggtgt	gttttgaca	cttatctcta	ttttaattga	tgtttttac	1380
tcgataaaaa	gtgtaaaaaa	caatttattt	ttttgtat	ttgttgttta	aaataattat	1440
agataatttt	atggttataa	attatttcat	taaacagtta	gcatctatgc	tctctaaatt	1500
tgagcatgt	ctagtagaca	ctttaaactt	ttaaacaat	agacacat	ctacctagca	1560
taatacacat	gtgatgcaac	aacgtaggca	caaattgcac	gttggctgga	tggcgtgcct	1620
actataagta	ttgaatttta	ccatagctt	ccatctacat	caaacttaaa	ggaaag	1676

<210> SEQ ID NO 40
<211> LENGTH: 1676
<212> TYPE: DNA
<213> ORGANISM: *S.tuberosum*
<220> FEATURE:
<223> OTHER INFORMATION: 23ACT promoter

<400> SEQUENCE: 40

ttagatcca	tgactcaatt	tgagaatgc	aaagagctcc	60		
tccgttcgc	ttctccaatt	acaataatca	agaatggaga	aatatagcaa	caatcgaaa	120

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<210> SEQ ID NO 41
<211> LENGTH: 1445
<212> TYPE: DNA
<213> ORGANISM: Solanum chacoense
<220> FEATURE:
<223> OTHER INFORMATION: 23DOX promoter
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<400> SEQUENCE: 41

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gggtccgact ttttgtttt aagaaatgc acattcgaag gatattttg tccaaagtgg 60
aaatattgtg taaaaaatgt gtatttattg tgattttgaa tgttttagt ggtaatagga 120
cgcacgcaaa gttaagggtgt cttttgaaa atcttgaca acttcaagta tcacttaatg 180
ttttttctca aaatctaatt tactttcgta aactatgtgt cagatcaaaa tttaacaaat 240
tcaaaaggaa tgagtaataa atagctctta caaatcacca ttttcgtct tcttcacact 300
caaaattttt ctcaatgaaa aaatgtgatt gattataatt agctcttaca catacttttg 360
ggttcttattt atttagattt gataaaaaga tatatcaaga aaacttcaaa tagaaaaaca 420
atataataag taaattcaag caatagatct tgaaaaatta aattttaaaa aaggtaatg 480
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agatattttt ttaacaaaag ctttttatta tgatttatgat gatgtagtca aagcagaaga	540
aaagaaaaagg gggtgtggtgg ggggtctggg gctgaaaaga agaggaaaaa ggtggggctg	600
ggggctgggc taaagtaagg ctggcaatgg aaccgggagg taccggtatac ggttcgtac	660
ctcccggttc cgatccgtta ccggctccat ctcggttccg gatagtaccc gttcgatccg	720
gtatttggtc ggtctggag gggtaacggg acggaaactg ggatttaccc gtctgtcccg	780
gtcccggttag tcccggttcc agtccgggtc gttcagtttt tttttttaa tcttaaattt	840
taaattatag ccgttgagag ccattgagca acgggttttgg gcgcacca acggctctt	900
cacccccata actccccctt ataattaata ataaggataa atttactaat taaatgtaaa	960
attattcatt aatttcataa aattgacaag tattattgaa catctcaaaa taatatagtg	1020
aacaactact ccatcagttcc ctatgtcc aattatccc tttcacttgt ggattaaatc	1080
aaaaaaagaat aaattttcc ctgttatgt atcgattaat taatttgaaa aagatagaac	1140
ttcttaaaaa tttaatattt ttatttctc tattttataa ttaataggga taaatttatta	1200
aatccactat gtcaaactact gtattttaa taaatatgtt aattttaaag caaatggaaat	1260
atataagacca catatccct cattttaaa gtaaaaatct tgatatttgt tcacatgatg	1320
aaatagttca cacccatttc ccattatata tagaccacca atttccatcat tttaaagta	1380
aaaacttaaa aagtgtttct tcttttttt ttctctttgc tcatattcta aaaagtattt	1440
catca	1445

<210> SEQ ID NO 42
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 42

gggtccgact ttttgggg 20

<210> SEQ ID NO 43
 <211> LENGTH: 20
 <212> TYPE: DNA
 <213> ORGANISM: Artificial Sequence
 <220> FEATURE:
 <223> OTHER INFORMATION: Artificially synthesized primer sequence

<400> SEQUENCE: 43

caatggcaat tgtggatca 20

<210> SEQ ID NO 44
 <211> LENGTH: 1592
 <212> TYPE: DNA
 <213> ORGANISM: S.chacoense
 <220> FEATURE:
 <223> OTHER INFORMATION: 23DOX promoter A

<400> SEQUENCE: 44

gggtccgact ttttgggg aagaaattgc acattcgaag gatatttttg tccaaagtgg	60
aaaatattgtg taaaaaatgt gtatttttg tgattttgaa tgttttgttag ggtaatagga	120
cgcacgc当地 gtttgggtgt cttttgaaa atttgggaca acttcaagta tcacttaatg	180
ttttttctc aaaatctaat ttactttgtt aaactatgag tcaatcaaaa atttaacaaa	240
ttaaaaaggaa atgagtaata aatagcttttcc accaaatcacc attttccatc ttcttcacac	300
tcaaaaatttt tctcaatgaa aaaatgtgtat tgattataat tagctttcac acataactttt	360

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<210> SEQ ID NO 45
<211> LENGTH: 1593
<212> TYPE: DNA
<213> ORGANISM: *S.chacoense*
<220> FEATURE:
<223> OTHER INFORMATION: 23DOX promoter B

<400> SEQUENCE: 45
gggtccgact ttttgtttt aagaaattgc acattcgaag gatattttg tccaaagtgg 60
aaatattgtg taaaaaatgt gtatttattg tgattttgaa tgttttgttag ggtaatagga 120
cgcacgcaaa gttaaggtgt cttttgaaa atctggaca acttcaagta tcacttaatg 180
tttttctca aaatctaatt tacttcgta aactatgtg cagatcaaaa tttaacaaat 240
tcaaaaggaa tgagtaataa atagtcctta caaatcacca ttttgcact tcttcacact 300
caaattttt ctcaatgaaa aaatgtgatt gattataatt agctcttaca catactttg 360
ggttctattt atttagattt gataaaaaga tatatcaaga aaacttcaaa tagaaaaaca 420
atataataag taaattcaag caatagatct tgaaaaatta aattttaaaa aaggtgaatg 480
agatattttt ttaacaaaag cttttattt tgattatgtat gatgttagtca aagcagaaga 540
aaagaaaaagg gggtggttgg ggggtctgga gctgaaaaga agagggaaaaa ggtggggctg 600
ggggctgggc taaagtaagg ctggcaacgg aatcgggagg taccggatc ggttccgatc 660
cgttaccggc tccatctcggttccggatag taccggatc ggttccgatc ggttccgatc 720
ggggggggta acggggacgga aactgggatt taccggatc ggttccgatc ggttccgatc 780

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gttcccgatcc gggtcggtca gttttttttt ttaatcttaa attttaaattt atagccgttg	840
agagccatttgg agcaacgggt ttggggcgcc accaacggct ctttcacccc cataactccc	900
ctttataattt aataataagg ataaattttac taattaaatg taaaatttttattt cattaatttc	960
ataaaaatttga caagtatttat tgaacatctc aaaataatat agtgaacaac tactccatca	1020
gtcccttattt gtccaatttt ccctttcac ttgtggatta aatcaaaaaa gaataaaattt	1080
tccctgtta tgctatcgat taattttttt gaaaaagata gaacttctta aaaattttaaa	1140
tttttttattt tctcttattttt ataaatttata gggataaattt attaaatccca ctatgtcaaa	1200
tactgttattt ttaataaaata tgttaatttta aaagcaaatttgaatatttggtaaagg	1260
agtaaaaaatc ttgatatttg ttcacatgtt gaaatagtgc acaccatc cccatttat	1320
atagaccaca tattttccatca tttttaaatgtt aaaaatcttgc atattttgttc acatgtatgt	1380
atagtagctt cacacccatttccatgtata tagaccac atattttccatca tttttaaatgtt	1440
taaaaatctt gatattttgtt cacatgtatgtt aatagtttc acccatttcc cattatatat	1500
agaccacatca tttccatcatca tttttaaatgtt aaaaatcttgc atattttgttc tttttttttt	1560
ctttttgttttctt atattttttttt aatgttttttttca	1593

<210> SEQ ID NO 46
<211> LENGTH: 32
<212> TYPE: DNA
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: Artifically synthesized primer sequence

<400> SEQUENCE: 46
taaaattatc gattaatttc ataaaaattdg a 32

<210> SEQ ID NO 47
<211> LENGTH: 955
<212> TYPE: DNA
<213> ORGANISM: *S.tuberosum*
<220> FEATURE:
<223> OTHER INFORMATION: 23DOX promoter A

<400> SEQUENCE: 47

taaaattattt cattaatttc ataaaattga caaatattgt tgaacatctc aaaataatat 60
agtggacaac tactccatcc atcccttattt gtccaaattt tccttttat ttgtcttattt 120
tcacaaatca agaaaagaata atttttccg tattatcca ttgattaatt aatttgaaaa 180
agatagaact tcttgaaaat tttaaatttt tatttctcta ctttataatt aataggaata 240
aatttattaaa cccactatgt caaatattat attttaata aatatgttaa tttaaaagca 300
gtaatttagaa ccgagggaaat attgttgaag ggatggagta aaaatcttga tatttgcgtt 360
agggttttgc cctgggttttc ttaccataat ttgagattta tttttctta aagaaaagac 420
aaaacactac cataaatgtt tttactttt ctgaaagaaa aatataatat tctttcatat 480
ttggtttattt ctccctttt aggaaaatgt ttatggagta gctagttctt ttcttaggg 540
aaaggtttta ggactctata aatatagtt tgttcttctt aacacaataa taacaataac 600
atccacaatg tagttttta agaatttagt ttatgaggag attttctcct aaacatattt 660
atgtttttta atagtagttt tcaatatgta ggtcggttga ccaaccata ttaataatat 720
atcttttagta tgtttttattt tatcgcttgtt tttgtcaacc atatgatttgc caattgtacg 780
cttccgcatg acgccctatt cacttcatg acccaacaat ttgttcacat gatgaaataa 840
ttcacccattt tccccattata tatagaccac ctatccctc attttaagt aaaaacttgc 900

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aagtgttct tcttttttt ttcttttgt tcataattcta aaaaatattt catca	955
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<210> SEQ ID NO 48
<211> LENGTH: 955
<212> TYPE: DNA
<213> ORGANISM: S.tuberosum
<220> FEATURE:
<223> OTHER INFORMATION: 23DOX promoter B

<400> SEQUENCE: 48

taaaattatt cattaatttc ataaaattga caaatattgt tgaacatctc aaaatagtat	60
agtggacaac tactccatcc atccctattt gtccaatttt tccttttat ttgtctattt	120
tcacaaatca agaaaagaata atttttccg tattatgccca ttgattaatt aatttgaaaa	180
agatagaact tcttggaaaat tttaaatttt tatttctcta ctttataatt aataggaaata	240
aattttaaaa cccactatgt caaatattat attttaataa aatatgttaa tttaaaagca	300
gtaatttagaa ccgagggaaat actgttgaag ggatggagta aaaatcttga tatttgcgtt	360
agggtttgc cctgggtttc ttaccataat ttaagattt ttttttotta aagaaaagac	420
aaaacactac cataaatgtt tttaacttttctgaaagaaaa aatataatat tctttcatat	480
ttggttttatt ctttcctttt aggaaaatgt ttatggagta gctagttttt ttcttagtagg	540
aaagggtttta ggactctata aatatagggtt tgtttcttct aacacaataa taacaataac	600
atccacaatg tagttttta agaatttagt ttatggggag attttctcct aaacatattt	660
atgctttta atagtaggtt tcaatatgtt ggtcggttgc ccaaaccata ttaataatat	720
atcttttagtg tgttttatt tategtctga ttgtcaacc atatgatttgc caattgtacg	780
cttcgcgtat acggccattt cacccatata acccaacaat ttgttcacat gatgaaatag	840
ttcacccatt tcccattata tatagaccac ctatttcctc attttaagt aaaaactttg	900
aagtgttct tcttttttt ttcttttgt tcataattcta aaaaatattt catca	955

<210> SEQ ID NO 49
<211> LENGTH: 955
<212> TYPE: DNA
<213> ORGANISM: S.tuberosum
<220> FEATURE:
<223> OTHER INFORMATION: 23DOX promoter A

<400> SEQUENCE: 49

taaaattatt cattaatttc ataaaattga caaatattgt tgaacatctc aaaataatat	60
agtggacaac tactccatcc atccctattt gtccaatttt tccttttat ttgtctattt	120
tcacaaatca agaaaagaata atttttccg tattatgccca ttgattaatt aatttgaaaa	180
agatagaact tcttggaaaat tttaaatttt tatttctcta ctttataatt aataggaaata	240
aattttaaaa cccactatgt caaatattat attttaataa aatatgttaa tttaaaagca	300
gtaatttagaa ccgagggaaat attgttgaag ggatggagta aaaatcttga tatttgcgtt	360
agggtttgc cctgggtttc ttaccataat ttgagattt ttttttotta aagaaaagac	420
aaaacactac cataaatgtt tttaacttttctgaaagaaaa aatataatat tctttcatat	480
ttggttttatt ctttcctttt aggaaaatgt ttatggagta gctagttttt ttcttagtagg	540
aaagggtttta ggactctata aatatagggtt tgtttcttct aacacaataa taacaataac	600
atccacaatg tagttttta agaatttagt ttatggggag attttctcct aaacatattt	660
atgctttta atagtaggtt tcaatatgtt ggtcggttgc ccaaaccata ttaataatat	720

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atcttttagta tgaaaaattt tatacgatgt ttgtcaacc atatgatttg caattgtacg	780
cttcggatg acggccattt cacccatgt acccaacaat ttgttcacat gatggaaataa	840
ttcacccatt tccccattata tatagaccac ctatccctc attttaagt aaaaacttg	900
aagtgtttct tctttttttt ttcttttgt tcatattcta aaaaatattt catca	955

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The invention claimed is:

1. A transformed cell that is capable of regenerating *Solanum tuberosum* having increased resistance against Colorado potato beetle, the transformed cell comprising:
 - (a) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6; and
 - (b) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12.
2. A transformed *Solanum tuberosum* plant having increased resistance to Colorado potato beetle, which is regenerated from the transformed cell according to claim 1.
3. A method for producing a *Solanum tuberosum* plant having Colorado potato beetle resistance, the method comprising:
 - introducing, to a cell, at least one DNA selected from the group consisting of (a) to (b) below and at least one DNA selected from the group consisting of (c) to (d) below; and
 - regenerating a *Solanum tuberosum* plant from a transformed cell to which the DNA selected from (a) to (b) and the DNA selected from (c) to (d) is introduced, wherein the DNAs are:
 - (a) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6;
 - (b) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6, and has an activity to hydroxylate position 23 of a spirostanol skeleton;
 - (c) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12; and
 - (d) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12, and has an activity to acetylate a hydroxy group of position 23 of a spirostanol skeleton.
4. A method for producing a *Solanum tuberosum* plant having resistance against Colorado potato beetle, the method comprising:
 - crossing a *Solanum tuberosum* plant having at least one DNA selected from the group consisting of (a) to (b)

below, with a *Solanum tuberosum* plant having at least one DNA selected from the group consisting of (c) to (d) below;

determining the resistance against Colorado potato beetle in a *Solanum tuberosum* plant obtained in the crossing, and

selecting a *Solanum tuberosum* plant that is determined to have the resistance to Colorado potato beetle, wherein the DNAs are

- (a) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6;
- (b) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 2, 4, or 6, and has an activity to hydroxylate position 23 of a spirostanol skeleton;
- (c) DNA encoding a protein that consists of an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12; and
- (d) DNA encoding a protein that consists of an amino acid sequence having 80% or higher identity to an amino acid sequence as set forth in SEQ ID NO: 8, 10, or 12, and has an activity to acetylate a hydroxy group of position 23 of a spirostanol skeleton.

5. The transformed cell that is capable of regenerating into a *Solanum tuberosum* plant having increased resistance against Colorado potato beetle according to claim 1, wherein the transformed cell has 1.1 times or higher expression of endogenous DNA-of (a) compared to an untransformed plant cell or has 1.1 times or higher expression of RNA corresponding to (a) compared to an untransformed plant cell, and

the transformed cell has 1.1 times or higher expression of endogenous DNA of (b) compared to the untransformed plant cell or has 1.1 times or higher expression of RNA corresponding to (b) compared to the untransformed plant cell.

6. The production method according to claim 3, wherein the *Solanum tuberosum* plant has an increased accumulation amount of leptine.

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