

Mar. 08, 2022

Bernadette Juarez
APHIS Deputy Administrator
Biotechnology Regulatory Services

Re: RSR submission No. 22-054-01rsr

Dear Dr. Bernadette Juarez,

This is our RSR request 22-054-01rsr that includes the requested additional information for walnut (*Juglans*) hybrid rootstocks that are resistant to crown gall disease by naturally occurring strains of the bacterial pathogen *Agrobacterium tumefaciens*. We greatly appreciate your continued thoughtful review of our request. Best Regards.

Sincerely,

Abhaya M. Dandekar
Distinguished Professor

Regulatory Status Review (RSR) Request for *Juglans* hybrid rootstock resistant to the formation of crown gall disease by natural strains of *Agrobacterium tumefaciens*.

1. Information about Requestor

First Name: Abhaya

Last Name: Dandekar

Position: Professor

Organization Name: University of California Davis

Contact information:

Telephone 530-752-7784

Email address amdandekar@ucdavis.edu

2. Does the request contain Confidential Business Information (CBI) - No

3. Description of the comparator plant:

Scientific name: *Juglans regia* x *Juglans hindsii* (hybrid seedling)

Common Name: Paradox

Subspecies: J1a-1A

4. Genotype of the modified plant.

A. If genetic material is inserted into the genome: Yes

Somatic embryos for J1a Paradox seedling were transformed with a 15,240 bp T-DNA from the binary vector pDE00.0201 to develop resistance to crown gall formation in rootstock plants derived from such embryos.

Position in T-DNA	Name of Sequence	Organism	Function of Sequence
1-308 (308)	LB	<i>Agrobacterium tumefaciens</i>	T-DNA left border
309-1,015 (707)	mas 5'	<i>Agrobacterium tumefaciens</i>	Selection marker promoter
1,016-1810 (795)	<i>npt II</i> /APH3'II	<i>Escherichia coli</i>	Kanamycin selection marker gene for transformation
1,811-2,677 (867)	mas 3'	<i>Agrobacterium tumefaciens</i>	selection marker terminator
2,678-2,935(258)	spacer	<i>Escherichia coli</i>	Non-coding spacer DNA
2,936-3,931 (996)	35S 5'	Cauliflower Mosaic Virus	ipt promoter
3,932-4,558 (627)	<i>ipt</i> sense	<i>Agrobacterium tumefaciens</i>	ipt sense coding sequence
4,559-5,546 (988)	linker	<i>Escherichia coli</i>	ipt non-coding linker sequence
5,547-6,173 (627)	<i>ipt</i> antisense	<i>Agrobacterium tumefaciens</i>	ipt antisense coding sequence
6,174-6,956 (783)	ocs3'	<i>Agrobacterium tumefaciens</i>	ipt terminator
6,957-7,993 (1,037)	35S 5'	Cauliflower Mosaic Virus	iaaM promoter
7,994-11,126 (3,133)	<i>iaa M</i> sense-antisense	<i>Agrobacterium tumefaciens</i>	iaaM sense and antisense coding sequence
11,127-11,906 (780)	ocs 3'	<i>Agrobacterium tumefaciens</i>	iaaM terminator
11,907-12,197 (291)	<i>nos</i> 3'	<i>Agrobacterium tumefaciens</i>	GUS terminator
12,198-14,009 (1,812)	<i>uid A</i> /GUS	<i>Escherichia coli</i>	β -glucuronidase coding sequence – a scorable marker gene for transformation
14,010-14,939 (930)	<i>ubi</i> 3 5'	<i>Solanum tuberosum</i>	Ubi3 promoter
14,940-15,240 (301)	RB repeat	<i>Agrobacterium tumefaciens</i>	T-DNA right border

308 bp sequence containing a left border (LB) region of T-DNA fragment from *Agrobacterium tumefaciens* component of GenBank: CP033030.1:

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CGGCAGGATATATTCAATTGTAAATGGCTTCATGTCCGGGAAATCTACATGGATCAGCAAT
GAGTATGATGGTCAATATGGAGAAAAAGAAAGAGTAATTACCAATTTTTTTTCAATTCAAAA
TGTAGATGTCCGCAGCGTTATTATAAAATGAAAGTACATTTTGATAAAACGACAAATTACGA
TCCGTCGTATTTATAGGCGAAAGCAATAAACAAATTATTCTAATTCGGAAATCTTTATTTTCA
CGTGTCTACATTCACGTCCAAATGGGGGCTTAGATGAGAACTTCACGATCGCCTCGAG
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707 bp sequence containing the mannopine synthase (*mas* 5') transcriptional regulatory sequence from *Agrobacterium tumefaciens* component of GenBank: CP033030.1:

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GGGCAACAGAGCCTGGCGTTCCCTTTTGCATTGAGACCGATGTTTCGTTCCGGAACCTT
GCACGCCCCAGAGCTTCTCACCGTTACGACAATTTCTTCTCGTTGAGGTCGGTCGCGC
CATGTCGGATGAAATAAAAATTTTTGATACCAGCGGGGGCCTTCGCAGAGCCGAGGTAGG
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TCTGAGAAATTGGCATTTCACGTGTGGAAGATATGAATTTTTTTGAGAACTAGATAAGAT
TAATGAATATCGGTGTTTTGGTTTTTTCTTGTGGCCGTCTTTGTTTATATTGAGATTTTTCAA
ATCAGTGCGCAAGACGTGACGTAAGTATCTGAGCTAGTTTTTATTTTTCTACTAATTTGGTC
GTTTATTTTCGGCGTGTAGGACATGGCAACCGGGCCTGAATTTTCGCGGGTATTCTGTTTCTA
TTCCAACTTTTTTCTTGATCCGCAGCCATTAACGACTTTTTGAATAGATACGCTGACACGCCAA
GCCTCGCTAGTCAAAGTGTACCAAACAACGCTTTACAGCAAGAACGGAATGCGCGTGAC
GCTCGCGGTGACGCCATTTTCGCCTTTTTCAGAAATGGATAAATAGCCTTGCTTCCTATTATAT
CTTCCCAAATACCAATACATTACACTAGCATCTGAATTTCATAACCAATCTCGATACACCCAAA
TCGACTCTAGCGAATTCCCCGGATCGTTTTCGC

795 bp synthetic sequence encoding the aminoglycoside phospho hydrolase 3 (APH (3') II)
coding region from *E. coli* Tn5 that confers resistance to the antibiotic kanamycin
similar/identical to GenBank: QCS90149.1:

ATGATTGAACAAGATGGATTGCACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTC
GGCTATGACTGGGCACAACAGACAATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCA
GCGCAGGGGCGCCCGTTCTTTTTGTCAAGACCGACCTGTCCGGTGCCCTGAATGAACTG
CAGGACGAGGCAGCGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGT
GCTCGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGC
AGGATCTCCTGTCATCCCACCTTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAAT
GCGGCGGCTGCATACGCTTGATCCGGCTACCTGCCATTCGACCACCAAGCGAAACATCG
CATCGAGCGAGCACGTACTIONCGGATGGAAGCCGGTCTTGTCGATCAGGATGATCTGGACGA
AGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCCGCCAGGCTCAAGGCGCGCATGCCCG
ACGGCGAGGATCTCGTCGTGACCCATGGCGATGCCTGCTTGCCGAATATCATGGTGGAAA
ATGGCCGCTTTTTCTGGATTCATCGACTGTGGCCGGCTGGGTGTGGCGGACCGCTATCAGG
ACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTGCGGGCGAATGGGCTGACCGCT
TCCTCGTGCTTTACGGTATCGCCGCTCCCGATTTCGACGCGCATCGCCTTCTATCGCCTTCT
TGACGAGTTCTTCTGA

867 bp sequence containing the mannopine synthase (*mas 3'*) 3' regulatory sequence from *A.*
tumefaciens component of GenBank: CP033030.1:

GCGGGACTCTGGGGTTCGAAATGACCGACCAAGCGACGCCAACCTGCCATCACGAGATT
TCGATTCACCGCCGCCTTCTATGAAAGTTGGGCTTCGGAATCGTTTTCCGGGACGCCG
GCTGGATGATCCTCCAGCGCGGGGATCTCATGCTGGAGTTCTTCGCCACCCCGGAATTC
CAGCTTCCCTGAAACCTTGGACTCCCATGTTGGCAAAGGCAACCAAACAATGAATGA
TCCGCTCCTGCATATGGGGCGGTTTGAGTATTTCAACTGCCATTTGGGCTGAATTGAAGAC
ATGCTCCTGTCAGAAATTCGGTATCTTACTCAATATTCAGTAATCTCGGCCAATATCCTAA
ATGTGCGTGGCTTTATCTGTCTTTGTATTGTTTCATCAATTCATGTAACGTTTGCTTTTCTTA
TGAATTTTCAAATAAATTATCGATAGTACTACGAATATTTTCGATCGCTGATCTTCTCAATCA
CAATGATGCGTAGTGACCCGACAAATAATTTAAGCGTCCTTAATACCAATCCTAAAATAATT
GAGGCAAATAAAATTTTTTTGTAATTTTTATGATAGCAGATCGATTCTCCAGCAAGCCTGCA
ACAAAATATTGTGTATTTCTAAATAGATTTTATGATTTAAAATCCCGAGAAAGCAAAATTGCAT
TTAACAAAACAGTAATTTAGTACATTAATAAAAATTATGCTCAAACATTTCCATGAAATTA
CATAATTACATTTTACAACACAATATTTTGCATATTATTGCCTTTTCGCCAATTTAACACTGG
AAGCAATTAAGTAGGCCACACAGAGCGCTTTGCAACACTGACAACGCTTGCGGTTGGT
CGG

258 bp *E. coli* plasmid noncoding sequences:

CCTCGAGGAGCTTGCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTCATTAAT
GCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAAT
GTGAGTTAGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGT

TGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACATGATTACGAA
TTCGAGCTCGGTACCTA

996 bp sequence containing the cauliflower mosaic virus (CaMV35S 5') 5' transcriptional regulatory sequences component of NCBI Reference Sequence: NC_001497.2;

GGCGCGCCTGAGCTTAACAGCACAGTTGCTCCTCTCAGAGCAGAATCGGGTATTCAACAC
CCTCATATCAACTACTACGTTGTGTATAACGGTCCACATGCCGGTATATACGATGACTGGG
GTTGTACAAAGGCGGCAACAAACGGCGTTCCCGGAGTTGCACACAAGAAATTTGCCACTAT
TACAGAGGCAAGAGCAGCAGCTGACGCGTACACAACAAGTCAGCAAACAGACAGGTTGAA
CTTCATCCCCAAAGGAGAAGCTCAACTCAAGCCCAAGAGCTTTGCTAAGGCCCTAACAAAGC
CCACCAAAGCAAAAAGCCCACTGGCTCACGCTAGGAACCAAAGGCCCAGCAGTGATCCA
GCCCAAAGAGATCTCCTTTGCCCGGAGATTACAATGGACGATTTCTCTATCTTTACG
ATCTAGGAAGGAAGTTCGAAGGTGAAGGTGACGACACTATGTTCACTGATAATGAGAA
GGTTAGCCTCTTCAATTTAGAAAGAATGCTGACCCACAGATGGTTAGAGAGGCCCTACGCA
GCAGGTCTCATCAAGACGATCTACCCGAGTAACAATCTCCAGGAGATCAAATACCTTCCCA
AGAAGGTTAAAGATGCAGTCAAAGATTGAGGACTAATTGCATCAAGAACACAGAGAAAGA
CATATTTCTCAAGATCAGAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCATAAAC
CAAGGCAAGTAATAGAGATTGGAGTCTCTAAAAAGGTAGTTCCCTACTGAATCTAACGCCAT
GCATGGAGTCTAAGATTCAAATCGAGGATCTAACAGAAGTCCCGTGAAGACTGGCGAAC
AGTTCATACAGAGTCTTTTACGACTCAATGACAAGAGAAATCTTCGTCAACATGTGAGCAC
GACACTCTGGTCTACTCCAAAATGTCAAAGATACAGTCTCAGAGACCAAAGGGCTATTGAG
ACTTTCAACAAAGGATAATTTT

627 bp sequence from containing the coding region for the *A. tumefaciens* isopentenyl phosphotransferase (*ipt*) in a sense orientation component of GenBank: AE007871.2;

ATGGACCTGCATCTAATTTTCGGTCCAACCTTGCACAGGAAAGACGTCGACCGCGGTAGCTC
TTGCCAGCAGACTGGGCTTCCAGTCTTTTCGCTCGATCGGGTCCAATGTTGTCTCAGCT
GTCAACCGGAAGCGGACGACCAACAGTGGAGAAGTCAAAGGAACGAGCCGTCTATACCT
TGATGATCGGCCTCTGGTGAAGGGTATCATCGCAGCCAAGCAAGCTCATGAAAGGCTGAT
GGGGGAGGTGTATAATTATGAGGCCACGGCGGGCTTATTCTTGAGGGAGGATCTATCTC
GTTGCTCAAGTGCATGGCGCAAAGCAGTTATTGGAGTGCGGATTTTCGTTGGCATATTATT
CGCCGCGAGTTAGCAGACGAGGAGACCTTCATGAACGTGGCCAAGGCCAGAGTTAAGCA
GATGTTACGCCCCGCTGCAGGCCTTTCTATTATCCAAGAGTTGGTTGATCTTTGAAAGAG
CCTCGGCTGAGGCCATACTGAAAGAGATCGATGGATATCGATATGCCATGTTGTTTGCTA
GCCAGAACCAGATCACATCCGATATGCTATTGCAGCTTGACGCAGATATGGAGGATAAGTT
GATTAATGGGATCGCTCAGGAG

988 bp non-coding spacer sequence from *E. coli*:

GAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTAGAGGGCCCAAT
TCGCCCTATAGTGAGTCGTATTACAATCACTGGCCGTCGTTTTACAACGTCGTGACTGGG
AAAACCCTGGCGTTACCCAATTAATCGCCTTGCAGCACATCCCCCTTCGCCAGCTGGCG
TAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCAACAGTTGCGCAGCCTGAATGGCGA
ATGGACGCGCCCTGTAGCGGCGCATTAAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGT
GACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTTCCCTTCTTTCTC
GCCACGTTTCGCCGGCTTTCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGA
TTTAGTGCTTTACGGCACCTCGACCCCAAAAATTTGATTAGGGTGTGTTTACGTAAGT
GGCCATCGCCCTGATAGACGGTTTTTTTCGCCCTTTGACGTTGGAGTCCACGTTCTTTAATAG
TGGACTCTTGTTCAAAATGGAACAACACTCAACCCTATCTCGGTCTATTCTTTTATTATA
AGGGATTTTGGCGATTTTCGGCCTATTGGTTAAAAAATGAGCTGATTTAACAAAATTTAACG
CGAATTTTAAACAAAATTCAGGGCGCAAGGGCTGCTAAAGGAAGCGGAACACGTAGAAAGC
CAGTCCGCAGAAACGGTGTGACCCCGGATGAATGTGAGCTACTGGGCTATCTGGACAAG

GGAAAACGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGATAGCT
AGACTGGGCGGTTTTATGGACAGCAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTG
GTAAGGTTGGGAAGCCCTGCAAAGTAACTGGATGGCTTTCTTGCCGCCAAGGATCTGAT
GGCGCAGGGGATCAAGATCT

627 bp containing the coding region for the *A. tumefaciens* isopentenyl phosphotransferase (*ipt*) in an anti-sense orientation component of GenBank: AE007871.2:

ATGGACCTGCATCTAATTTTCGGTCCAACCTTGCACAGGAAAGACGTGACCGCGGTAGCTC
TTGCCAGCAGACTGGGCTTCCAGTCCTTTTCGCTCGATCGGGTCCAATGTTGTCCTCAGCT
GTCAACCGGAAGCGGACGACCAACAGTGGAAGAACTGAAAGGAACGAGCCGTCTATACCT
TGATGATCGGCCTCTGGTGAAGGGTATCATCGCAGCCAAGCAAGCTCATGAAAGGCTGAT
GGGGGAGGTGTATAATTATGAGGCCACGGCGGGCTTATTCTTGAGGGAGGATCTATCTC
GTTGCTCAAGTGCATGGCGCAAAGCAGTTATTGGAGTGCGGATTTTCGTTGGCATATTATT
CGCCGCGAGTTAGCAGACGAGGAGACCTTCATGAACGTGGCCAAGGCCAGAGTTAAGCA
GATGTTACGCCCCGCTGCAGGCCTTTCTATTATCCAAGAGTTGTTGATCTTTGGAAAGAG
CCTCGGCTGAGGCCATACTGAAAGAGATCGATGGATATCGATATGCCATGTTGTTTGCTA
GCCAGAACCAGATCACATCCGATATGCTATTGCAGCTTGACGCAGATATGGAGGATAAGTT
GATTAATGGGATCGCTCAGGAG

783 bp sequence containing the octopine synthase (*ocs* 3') 3' regulatory region from *A. tumefaciens* component of GenBank: CP033030.1:

GTCCTGCTTTAATGAGATATGCGAGACGCCTATGATCGCATGATATTTGCTTTCAATTCTGT
TGTGCACGTTGTAAAAAACCTGAGCATGTGTAGCTCAGATCCTTACCGCCGGTTTCGGTTC
ATTCTAATGAATATATCACCCGTTACTATCGTATTTTTATGAATAATATTCTCCGTTCAATTTA
CTGATTGTACCCTACTACTTATATGTACAATATTAATAATGAAAACAATATATTGTGCTGAATA
GTTTTATAGCGACATCTATGATAGAGCGCCACAATAACAAACAATTGCGTTTTATTATTACA
AATCCAATTTTAAAAAAGCGGCAGAACCGGTCAAACCTAAAAGACTGATTACATAAATCTT
ATTCAAATTTCAAAGTGCCCCAGGGGCTAGTATCTACGACACACCGAGCGGCGAACTAAT
AACGCTCACTGAAGGGAACCTCCGTTCCCGCCGCGCGCATGGGTGAGATTCTTGAAG
TTGAGTATTGGCCGTCGCTCTACCGAAAGTTACGGGCACCATTCAACCCGGTCCAGCAC
GGCGGCCGGGTAACCGACTTGCTGCCCGAGAATTATGCAGCATTTTTTTGGTGTATGTG
GGCCCAAATGAAGTGACAGGTCAAACCTTGACAGTGACGACAAATCGTTGGGCGGGTCCA
GGGCGAATTTTGCGACAACATGTCGAGGCTCAGCAGGACCTGCAGGCATGCAAGCTAGCT
TACTAGTGATGCATATTCTATAGTGTACCTAAATCTGCGGCCGCTGA

1037 bp sequence containing the cauliflower mosaic virus (CaMV 35S 5') transcriptional enhancer and promoter sequence component of NCBI Reference Sequence: NC_001497.2;

CTATAGGGCGATTGAATTTAGCGGCCGCGAATTCGCCCTTAGGCGCGCCTGAGCTTAACA
GCACAGTTGCTCCTCTCAGAGCAGAATCGGGTATTCAACACCCTCATATCACTACTACGT
TGTGTATAACGGTCCACATGCCGGTATATACGATGACTGGGGTTGTACAAAGGCGGCAAC
AAACGGCGTTCCCGGAGTTGCACACAAGAAATTTGCCACTATTACAGAGGCAAGAGCAGC
AGCTGACGCGTACACAACAAGTCAGCAAACAGACAGGTTGAACTTCATCCCCAAAGGAGA
AGCTCAACTCAAGCCCAAGAGCTTTGCTAAGGCCCTAACAAGCCACCAAGCAAAAAGC
CCACTGGCTCACGCTAGGAACCAAAAGGCCAGCAGTGATCCAGCCCCAAAGAGATCTC
CTTTGCCCGGAGATTACAATGGACGATTTCTCTATCTTTACGATCTAGGAAGGAAGTTC
GAAGGTGAAGGTGACGACACTATGTTCACTGATAATGAGAAGGTTAGCCTCTTCAATT
TCAGAAAGAATGCTGACCCACAGATGGTTAGAGAGGCCTACGCAGCAGGTCTCATCAAGA
CGATCTACCCGAGTAACAATCTCCAGGAGATCAAATACCTTCCAAGAAGGTTAAAGATGC
AGTCAAAAAGATTGAGGACTAATTGCATCAAGAACACAGAGAAAGACATATTTCTCAAGATCA
GAAGTACTATTCCAGTATGGACGATTCAAGGCTTGCTTCATAAACCAAGGCAAGTAATAGA
GATTGGAGTCTCTAAAAGGTAGTTCTACTGAATCTAACGCCATGCATGGAGTCTAAGATT

CAAATCGAGGATCTAACAGAACTCGCCGTGAAGACTGGCGAACAGTTCATACAGAGTCTTT
TACGACTCAATGACAAGAGAAAATCTTCGTCAACATGTGAGCACGACACTCTGGTCTACTC
CAAATGTCAAAGATACAGTCTCAGAGACCAAAGGGCTATTGAGACTTTCAACAAAGGATA
ATTTT

3133 bp sequence containing the coding region for the *Agrobacterium tumefaciens* tryptophan
2-monooxygenase (*iaaM*) coding sequence in sense fused to an antisense orientation
component of GenBank: AE007871.2:

ATGGGAATAGGATCTGGCGGGTTTGGTCCAGTTTTTCAAAGCGGGTTTATTGAGATCCTTC
GCTTGGTCATAAACGGATATGAAGAAAATCAGCGGATGTGCTCTGAAGGAATCTCAGAACT
TCCACGTGCAATAGCCACTCAAGTGGTTAACGGTGTGTCTGTAAGCCAGCGTATACGCCAT
GTTCAAGTCAGGGCGATTGAGAAGGAAAAGACAAAATAAAGATAAGGCTTAAGAGCGGG
ATATCTGAACTTTATGATAAGGTGGTGGTTACATCTGGACTCGCAAATATCCAACCTCAGGCA
TTGTCTGACATGCGATACCACCTTTTTCGTGCACCAGTGAACCAAGCGGTTGATAACAGC
CATATGACAGGCTCGTCAAACTCTTCCTGCTGACTGAACGAAAATTTTGGTTAGACCATAT
CCTCCCGTCCTGTGTCTCATGGACGGGATCGCAAAGCAGTGTACTGCCTGGACTATGA
GCCGCAGGATCCGAATGGTAAAGGTCTGGTGTCTCATCAGTTATACATGGGAGGACGACTC
CCACAAGCTGTTGGCGGTTCCCGACAAAAGAGCGATTCTGTCTGCTGCGGGACGCAAT
TTCGAGATCTTTCCCGGAGTTTCCCAGCATCTAGTTCCCGCCTGCGCTGATTACGACCAA
AATGTTGTTCAACATGATTGGCTTACAGACGAGAATGCCGGGGGAGCTTTCAAACCTCAACC
GGCGTGGCGAGGATTTTTATTCTGAAGAACTTTTCTTTCAAGCGCTGGACATGACTAATGAT
ACCGGAGTTTACTTGGCGGGTTGCAGTTGTTCCCTTACCAGGTGGATGGGTGGAGGGCGCT
ATTCAGACCGCGTGTAAACGCCGTCTGTGCAATTATCCACAATTGTGGAGGATTTTTGGCAA
AGGACAATCCTCTCGAACACTCTTGAAGAGATATAACTACCGCATAAGGCTGATGAATTG
GACCGCAGGGTTTCCGATGCCTTCTCAGAACGTGAAGCTTCTAGGGGAAGGAGGATTACT
CAAATCTCCGGCGAGTGCAGCGCTGGGTTAGCTTGCAAAGGCTGGCCGACGGTTCGCTTT
CCCGAGATCTCAGCTGGTGAAGGTAGCAGCCCTCTCCGCTTACATCTATGTTGGCAAG
GAAATTCTGGGGCGGATACTTGAATCGGAACCTTGGGCGCGAGCAAGAGTGAGTGGTCTC
GTTGCCATCGACCTTGCACCATTTGTATGGATTTCTCCGAAGCACAACCTTCTCCAAACCCT
GTTTTTGTGAGCGGTAAAAGATGTGCATCCACCGATCTTAGTCATTTCTGGCCATTTCAA
TCTCTAAGACTGCCGGTCCGAACCCTGCAAATGCCGCCGTACGAGAAAGCACGACGAAAC
GCGTTACCGGGTTTACCCTGACCCTTGAAGAGGCCCGTACCATTTGACATGGTAGCTTATG
GTCCGAAACCTGATGCTGAAGGCTTCCGGCAGGTTCTTTCCAACAATTGACTTACTCTATGA
CTACAGATCGTTTTTTGACCAATGTTCCGATAGTGGACGGATCGGCTTCTTTCCGGAAGAT
GTTCTTAAACCAAAAGTGGCGATCATTGGCGCTGGCATTTCGGGACTCGTGGTGGCAAGC
GAACTGCTTCATGCTGGTGTAGACAATGTTACAATATATGAAGCAAGTGATCGGGTTGGAG
GCAAGCTTTGGTCACATGCTTTCAAGGACGCTCCCAGCGTGGTGGCCGAAATGGGGGCGA
TGCGATTTCTCCTGCTGCATCGTGTGTTTTTTCTTTCTCGAGCGGTACGGCTGTCTTCGAT
GAGGCCGNTCCCAAATCCCGGCACAGTNGACACTAACTTGGTCTACCAAGGCCTCCGATA
CATGTGGAAAGCGGGCAGCAACCACCGAAGCTGTTCCATCGCGTTACAGCGGTTGGCGTG
CGTTCTTGAAGGACGGTTTCCATGAGGAGATATTGTGTTGGCTTCGCCTGTTGCTATTACT
CAAGCCTTGAATCAGAGACATTAGGCGGGCTCATGACTCCTGCAAACCTTGGGTGAACCG
TTCGGGAGGAGTCTTCTTTCAGCGATAGAGAGGATCTTTCTGGGCACGCATCCTCCTG
GTGGTGAACATGGAGTTTCCCTCATGATTGGGACCTATTCAAGCTAATGGGAATAGGATC
TGCCGGGTTTGGTCCAGTTTTTCAAAGCGGGTTTATTGAGATCCTTCGCTTGGTCATAAAC
GGATATGAAGAAAATCAGCGGATGTGCTCTGAAGGAATCTCAGAACTTCCACGTGCAATAG
CCACTCAAGTGGTTAACGGTGTGTCTGTAAGCCAGCGTATACGCCATGTTCAAGTCAGGG
CGATTGAGAAGGAAAAGACAAAATAAAGATAAGGCTTAAGAGCGGGATATCTGAACTTTA
TGATAAGGTGGTGGTTACATCTGGACTCGCAAATATCCAACCTCAGGCATTGTCTGACATGC
GATACCACCTTTTTTTCGTGCACCAGTGAACCAAGCGGTTGATAACAGCCATATGACAGGCT
CGTCAAACTCTTCCTGCTGACTGAACGAAAATTTTGGTTAGACCATATCCTCCCGTCCTGT

GTCCTCATGGACGGGATCGCAAAGCAGTGTACTGCCTGGACTATGAGCCGCAGGATCCG
AATGGTAAAGGTCTGGTGCTCATCAGTTATACATGGGAGGACGACTCCCACAAGCTGTTGG
CGGTTCCCGACAAAAAAGAGCGATTCTGTCTGCTGCGGGACGCAATTTGAGATCTTTCCC
GGAGTTTGCCAGCATCTAGTTCCCGCCTGCGCTGATTACGACCAAATGTTGTTCAACAT
GATTGGCTTACAGACGAGAATGCCGGGGAGCTTTCAAACTCAACCGCGTGGCGAGGAT
TTTTATTCTGAAGAACTTTTTCTTTCAAGCGCTGGACATGACTAATGATACCGGAGTTTACTT
GGCGGGTTGCAGTTGTTCTTCACCGGTGGATGGGTGGAGGGCGCTATTCAGACCGCGT
GTAACGCCGTCTGTGCAATTATCCACAATTGTGGAGGTATTTTGGCAAAGGACAATCCTCT
CGAACACTCTTGAAGAGATATAACTACCGCAGTC

780 bp sequence containing the 3' regulatory region of the octopine synthase (*ocs3'*) gene from *Agrobacterium tumefaciens* component of GenBank: CP033030.1:

CTGCTTTAATGAGATATGCGAGACGCCTATGATCGCATGATATTTGCTTTCAATTCTGTTGT
GCACGTTGTAACAACTGAGCATGTGTAGCTCAGATCCTTACCGCCGGTTTCGGTTCATT
CTAATGAATATATCACCCGTTACTATCGTATTTTTATGAATAATATTCTCCGTTCAATTTACTG
ATTGTACCCTACTACTTATATGTACAATATTTAAATGAAAACAATATATTGTGCTGAATAGGT
TTATAGCGACATCTATGATAGAGCGCCACAATAACAAACAATTGCGTTTTATTATTACAAATC
CAATTTTAAAAAAGCGGCAGAACCGGTCAAACCTAAAAGACTGATTACATAAATCTTATTC
AAATTTCAAAGTGCCCCAGGGGCTAGTATCTACGACACACCGAGCGGCGAACTAATAAC
GCTCACTGAAGGGAACCTCCGGTTCCCGCCGCGCGCATGGGTGAGATTCTTGAAGTTG
AGTATTGGCCGTCGCTCTACCGAAAGTTACGGGCACCATTCAACCGGTCCAGCACGGC
GGCCGGGTAACCGACTTGCTGCCCGAGAATTATGCAGCATTTTTTTGGTGTATGTGGGC
CCCAAATGAAGTGCAGGTCAAACCTTGACAGTGACGACAAATCGTTGGGCGGGTCCAGGG
CGAATTTTGCGACAACATGTGAGGCTCAGCAGGACCTGCAGGCATGCAAGCTAGCTTAC
TAGTGATGCATATTCTATAGTGTACCTAAATCTGCGGCCGCTGA

291 bp reverse complimentary sequence of the 3' regulatory sequence of the nopaline synthase (*nos 3'*) gene from *Agrobacterium tumefaciens* component of GenBank: AE007871.2:

GCGGCCGCCCGGGGATCGATCCCCGATCTAGTAACATAGATGACACCGCGCGGATAATT
TATCCTAGTTTGCGCGCTATATTTTGTCTTCTATCGCGTATTAATGTATAATTGCGGGACTC
TAATCATAAAAACCCATCTCATAAATAACGTCATGCATTACATGTTAATTATTACATGCTTAA
CGTAATTCAACAGAAATTATATGATAATCTATCGCAAGACCGGCAACAGGATTCAATCTTAA
GAACTTTATTGCCAAATGTTTGAACGATCGGGAATTGGATCC

1812 bp reverse complimentary sequence of the coding region of the β -glucouronidase (*uidA* or *GUS*) gene from *E. coli* GenBank: S69414.1:

TCATTGTTTGCCTCCCTGCTGCGGTTTTTCACCGAAGTTCATGCCAGTCCAGCGTTTTTGCA
GCAGAAAAGCCGCCGACTTCGGTTTTGCGGTGCGGAGTGAAGATCCCTTTCTTGTTACCGC
CAACGCGCAATATGCCTTGCGAGGTGCGAAAATCGGCGAAATTCATACCTGTTACCGGAC
GACGGCGCTGACGCGATCAAAGACGCGGTGATACATATCCAGCCATGCACACTGATACTC
TTCCTCCACATGTCGGTGTACATTGAGTGCAGCCCGGCTAACGTATCCACGCCGTATTCCG
GTGATGATAATCGGCTGATGCAGTTTCTCCTGCCAGGCCAGAAGTTCTTTTTCCAGTACCT
TCTCTGCCGTTTCAAATCGCCGCTTTGGACATACCATCCGTAATAACGGTTCAGGCACAG
CACATCAAAGAGATCGCTGATGGTATCGGTGTGAGCGTTCGACAGAACATTACATTGACGCA
GGTATCGGACGCGTCGGGTGAGTTTACGCGTTGCTTCCGCCAGTGGCGCGAAATATTC
CCGTGCACCTTGCGGACGGGTATCCGGTTCGTTGGCAATACTCCACATCACCACGCTTGG
GTGGTTTTTGTACGCGCTATCAGCTCTTTAATCGCCTGTAAGTGCGCTTGCTGAGTTTCC
CCGTTGACTGCCTCTTCGCTGTACAGTTCTTTCCGGCTTGTTGCCCGCTTCGAAACCAATGC
CTAAAGAGAGGTTAAAGCCGACAGCAGGATTTTCATCAATCACCACGATGCCATGTTTCATC
TGCCAGTTCGAGCATCTTTCAGCGTAAGGGTAATGCGAGGTACGGTAGGAGTTGGCCCC
AATCCAGTCCATTAATGCGTGGTTCGTGCACCATCAGCACGTTATCGAATCCTTTGCCACGC

AAGTCCGCATCTTCATGACGACCAAAGCCAGTAAAGTAGAACGGTTTGTGGTTAATCAGGA
ACTGTTTCGCCCTTCACTGCCACTGACCGGATGCCGACGCGAAGCGGGTAGATATCACACT
CTGTCTGGCTTTTGGCTGTGACGCACAGTTCATAGAGATAACCTTACCCGGTTGCCAGAG
GTGCGGATTCACCACTTGCAAAGTCCCGCTAGTGCCTTGTCCAGTTGCAACCACCTGTTGA
TCCGCATCACGCAGTTCAACGCTGACATCACCAATTGGCCACCACCTGCCAGTCAACAGAC
GCGTGGTTACAGTCTTGC GCGACATGCGTCACCACGGTGATATCGTCCACCAGGTGTTCC
GGCGTGGTGTAGAGCATTACGCTGCGATGGATTCCGGCATAAGTTAAAGAAATCATGGAAG
TAAGACTGCTTTTTCTTGCCGTTTTCTGTCGGTAATCACCAATTCCCGGCGGGGATAGTCTGCC
AGTTCAGTTCGTTGTTACACAAAACGGTGATACGTACACTTTTCCCGGCAATAACATACGG
CGTGACATCGGCTTCAAATGGCGTATAGCCGCCCTGATGCTCCATCACTTCTGATTATTG
ACCCACACTTTGCCGTAATGAGTGACCGCATCGAAACGCAGCACGATACGCTGGCCTGCC
CAACCTTTCGGTATAAAGACTTTCGCGCTGATACCAGACGTTGCCCGCATAATTACGAATAT
CTGCATCGGCGAACTGATCGTTAAACTGCCTGGCACAGCAATTGCCCGGCTTTCTTGTA
CGCGCTTCCACCAACGCTGATCAATCCACAGTTTTCGCGATCCAGACTGAATGCCAC
AGGCCGTCGAGTTTTTTGATTTACAGGGTTGGGGTTTCTACAGGACGTGCCAT

930 bp reverse complimentary sequence of the potato 5' regulatory sequence for the ubiquitin 3
(ubi3 5') gene from *Solanum tuberosum* component of GenBank: CP055241.1;
CCCGGGGATCCTTCGCCTGGAGGAGAGAAATCAGTGGCGCTGCGGCTTTTAGGGTTTCTT
TGTTGATGGAATGAGAGTGTAAGCTCTGCCAGTGCCACTTTATTAGGGTTTTACAAGCCCT
TTTCTTCGTAATTGGGCTGACATTTTGTGCCACTTGGGCCTTTAGAGATGAAAATGTATAT
TGGGCTTAAGTTGACTTGAAGGATAAATTAGTTTAGGATATTACGTTTTTTATGAGAATTGGT
GTGTCGGATACATGTATATGATGCATTCAAATATATGATTCTAGATACATTTAAGTTTAGAT
ACAATCTAAAATGTGTCTTTAATTACAGGACTGTAATAAATACTTAATGTAAGAAGAATAT
TACTCCTTTAATAGCTTTTGTAGTATATCTAGTCTAACATCTTTTAAAAAAGTCTAATTTCTTTC
ATTTATTTTTTCGAGCAATAGCAAAGTGCATAATTATTTTTTTCTTCTAGAAATTCAGATTTGTT
TCTCTAAATTTTGTAGATTCTTTTCTCAATTTTGTATGTCTAGAGAACAATGTGTATTTTCACT
CTAGTTGGTTGTTGCTTTGTTGAATGTTCTGATAAAAGTATATTGTTATTTCTGAAGTAGATA
TAAACCTTCATTTGGAAATTATACATAAATCAAATCGTTAATTATCTAGATCAAGATATATG
CCCTTTTCCTAATGTATTTGATACATGCACCTAATTTCACTAGATGTATCTTTTCTATTTTTTA
AATTATGAATAGTTAATTTTTTCCATATGTGTATTTGATACATACTTCATGACTTTAAAAAATT
AATTATATACCAGATATATGTATTTAAATTTGTTATGTATTTAAAGTATGTATATGATTATTC
GATATTAATCTCTTCGATGAAATTTAAATCGATAAGTATGTGCTTTGG

301 bp sequence containing the right border (RB) sequence from *Agrobacterium tumefaciens*
component of GenBank: CP033030.1:
TTAATTAATCTAGAATTTAAATGGATCCGCGGCCGCCCTGCAGGAAGCTTGGCACTGGCCG
TCGTTTTACAACGTCGTGACTGGGAAAACCCTGGCGTTACCCAACCTAATCGCCTTGCAGC
ACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCA
ACAGTTGCGCAGCCTGAATGGCGAATGAGACCTCAATTGCGAGCTTTCTAATTTCAAATA
TTCGGGCCTAACTTTTGGTGTGATGATGCTGACTGGCAGGATATATACCGTTGTAATT

B. If genetic material is not inserted into the genome: N/A

5. Description of new trait

Intended trait: Resistance to crown gall formation by natural strains of *Agrobacterium tumefaciens*.

Intended phenotype: No gall/tumor, inhibition of the formation of crown galls or tumors at the site of infection.

Description of the MOA: The inserted T-DNA contains four transgenes two that are expressed in the rootstock to create double stranded RNA corresponding to the common and highly conserved *Agrobacterium* genes *ipt* and *iaaM* responsible for tumor formation (Escobar *et al.*, 2001; 2002; 2003). Typically, when wild-type walnut plants are infected with *Agrobacterium*, these genes are transferred from the pathogen into the infected cells (Escobar and Dandekar 2003a). Their expression in the host results in uncontrolled cell proliferation and the formation of Crown Gall Tumors and disease. When these two transgenes are expressed in plants the self-complementary mRNA forms a dsRNA that serves as a trigger to induce post transcriptional gene silencing mediated by siRNA (created from the dsRNA) that targets the degradation of *ipt* and *iaaM* mRNA in a sequence specific manner (Escobar and Dandekar 2003b). In this particular case since dsRNA are made for the *iaaM* and *ipt* coding sequences any incoming T-DNA that expresses these two genes that results in the synthesis of mRNA is targeted for degradation via a host induced RNAi-mediated gene silencing that provides resistance to the formation of crown gall tumors as has been previously described (Escobar *et al.* 2001, 2003 and Escobar and Dandekar 2003b). The T-DNA contains a third transgene sequence encoding the aminoglycoside phospho hydrolase 3 (APH (3') II) coding region that confers resistance to the antibiotic kanamycin, a phenotype that was used to select for transformed embryos as described by McGranahan *et al.*, 1988. The encoded APH (3') II enzyme when expressed inactivates the antibiotic kanamycin so it is no longer able to inhibit the growth of walnut embryos in culture (McGranahan *et al.*, 1988). The coding region for the fourth transgene encodes a β -glucouronidase (*uidA* or GUS) enzyme present near the right border of the T-DNA and is used as a visual screenable marker gene to improve the efficiency of walnut embryo transformation as described by McGranahan *et al.*, 1990. Transformed cells and embryos that express the GUS gene make the β -glucouronidase enzyme that can cleave a variety of glucuronide substrates, in particular X-Gluc (5-bromo-4-chloro-3 indolyl glucuronide) that release an indigo blue color histochemical stain in the tissues that is the phenotype used to screen transformed embryos (McGranahan *et al.*, 1990). Both genes are required and used in combination for the transformation and selection of transgenic walnut plants (McGranahan *et al.*, 1990).

Other information on MOA (when relevant).

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Escobar, M.A., Leslie, C.A., McGranahan, G.H., & Dandekar, A.M. 2002. Silencing crown gall disease in walnut (*Juglans regia* L.). *Plant Science* 163: 591-597.

Escobar, M.A., Civerolo, E.L., Polito, V.S., Pinney, K.A., & Dandekar, A.M. 2003. Characterization of oncogene-silenced transgenic plants: Implications for *Agrobacterium* biology and post-transcriptional gene silencing. *Molecular Plant Pathology* 4: 57-65.

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McGranahan, G.H., C.A. Leslie, S.L. Uratsu, L.A. Martin and A.M. Dandekar. 1988. *Agrobacterium*-mediated transformation of walnut somatic embryos and regeneration of transgenic plants. *Bio/Technology* 6: 800-804.

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