Small secreted proteins from the necrotrophic conifer pathogen *Heterobasidion annosum s.l.* (HaSSPs) induce cell death in *Nicotiana benthamiana*

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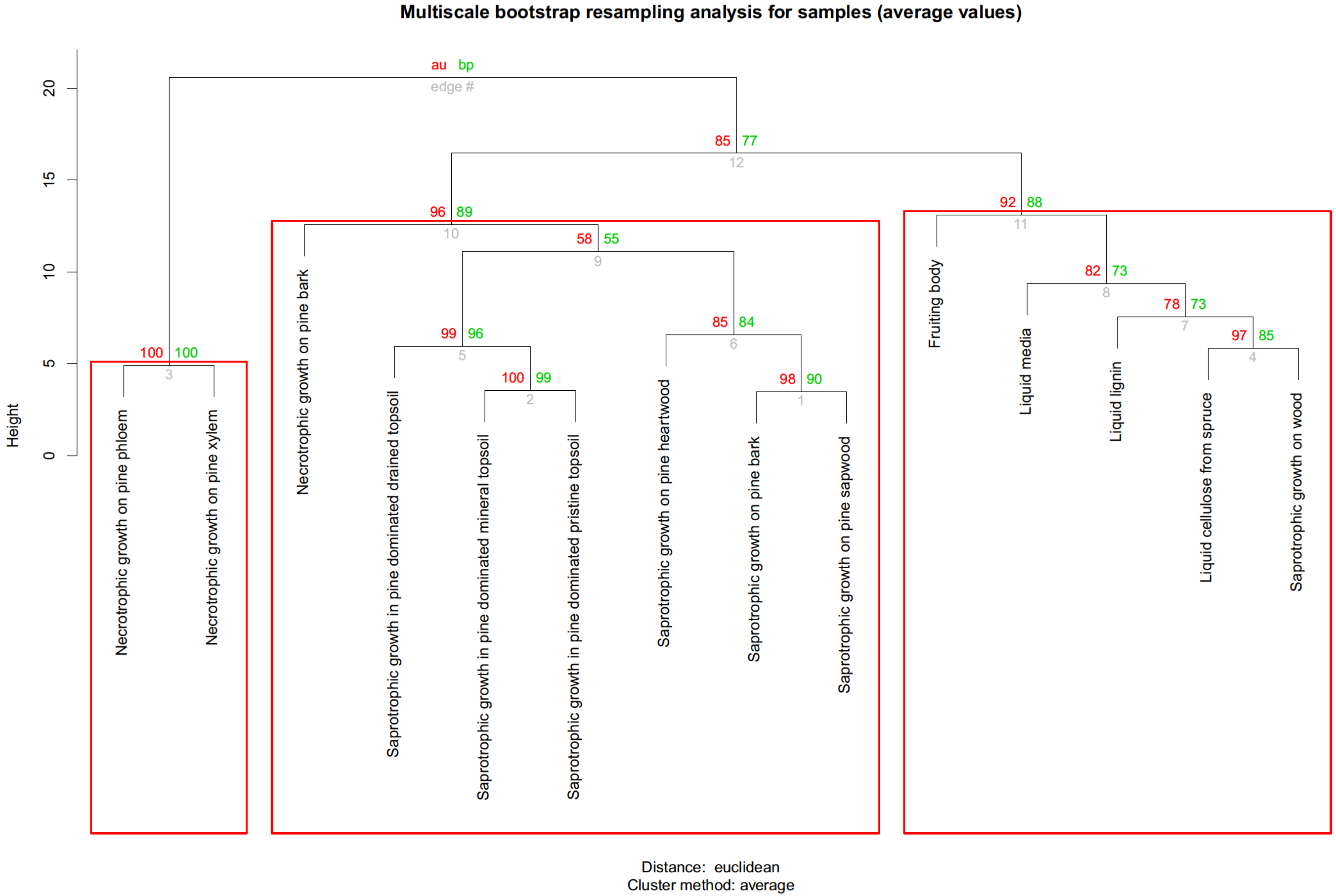
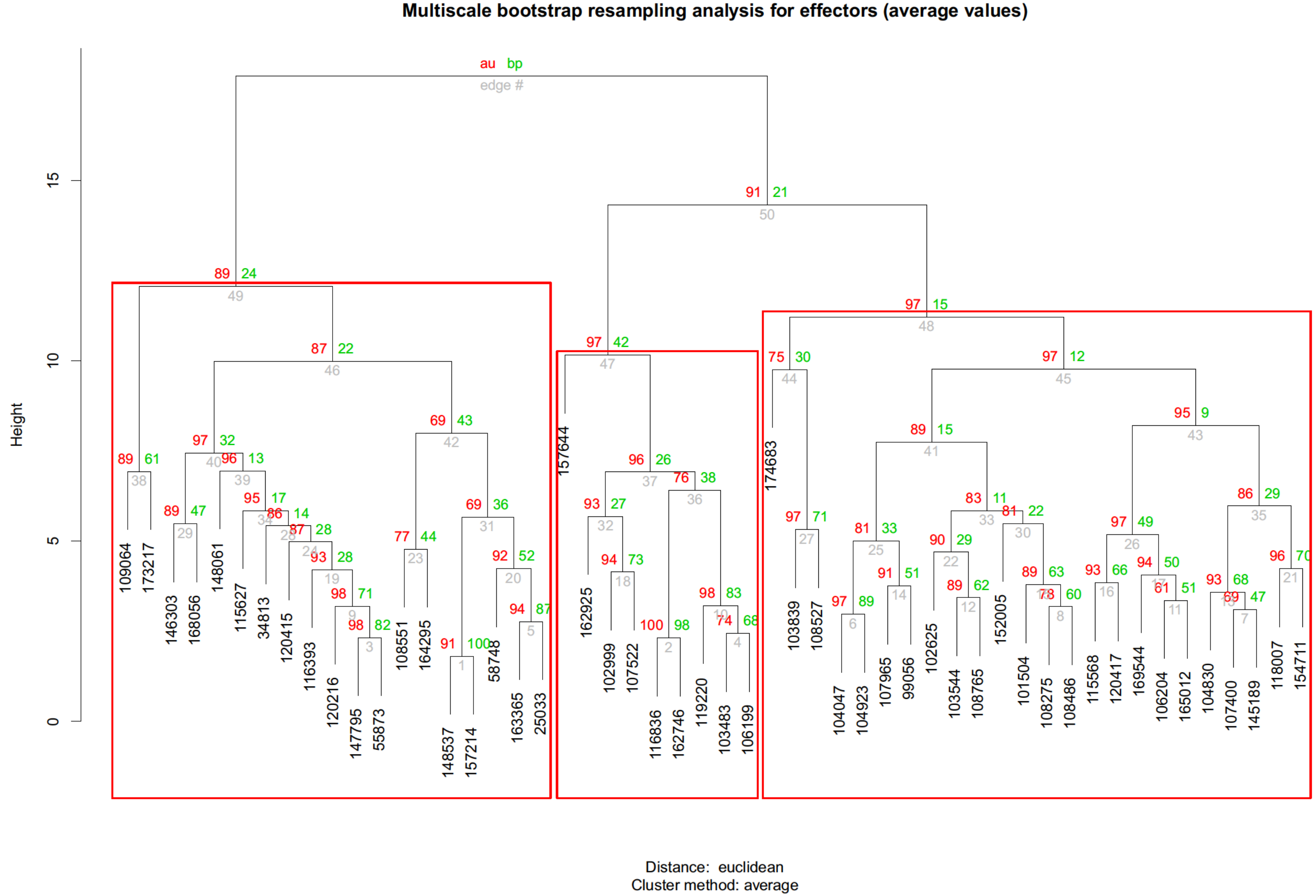
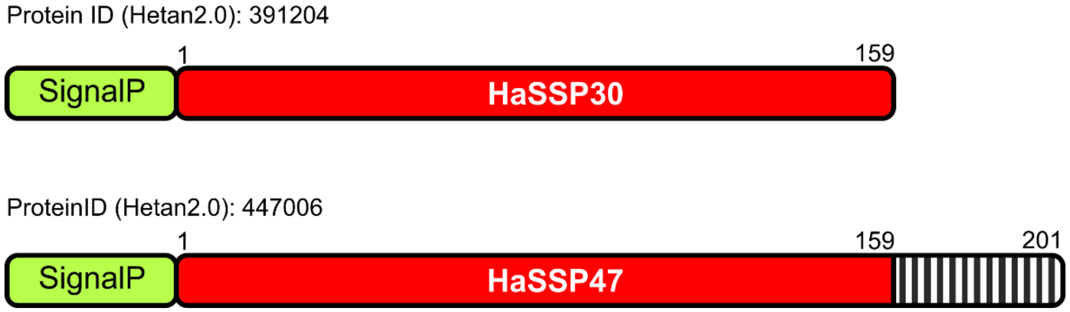
Supplementary Figure 1: Statistical analysis of the microarray sample data clustering by multiscale bootstrap resampling. Microarray data were retrieved from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/). Raw data were normalized with the statistical program R[48] using the *oligo* package[49]. The statistic *p-values*, "au" (approximately unbiased) and "bp" (bootstrap probability), for the sample clusters were calculated in R using the *pvclust* package in order to assess the statistical significance of the cluster analysis by multiscale bootstrap resampling[51].



Supplementary Figure 2: Statistical analysis of the microarray gene data clustering by multiscale bootstrap resampling. Microarray data was retrieved from the Gene Expression Omnibus database (GEO) (https://www.ncbi.nlm.nih.gov/geo/). Raw data was normalized with the statistical program R[48] using the *oligo* package[49]. The statistic *p-values*, "au" (approximately unbiased) and "bp" (bootstrap probability), for the gene clusters were calculated in R using the *pvclust* package in order to assess the statistical significance of the cluster analysis by multiscale bootstrap resampling[51].



Supplementary Figure 3: Comparison of HaSSP30 and HaSSP47 protein length.



Supplementary Table S1: List of the microarray samples analysed in this study from the Gene Expression Omnibus database (GEO).

Accession number in Gene Expression Omnibus (GEO) https://www.ncbi.nlm.nih.gov/geo/	Sample description[1]	Reference
GSM748496	necrotic bark of pines inoculated with Heterobasidion irregulare 1	(Olson, Aerts et al. 2012)
GSM748497	necrotic bark of pines inoculated with Heterobasidion irregulare 2	(Olson, Aerts et al. 2012)
GSM748498	necrotic bark of pines inoculated with Heterobasidion irregulare 3	(Olson, Aerts et al. 2012)
GSM748499	Heterobasidion irregulare grown in liquid medium amended with cellulose from Spruce 1	(Olson, Aerts et al. 2012)
GSM748500	Heterobasidion irregulare grown in liquid medium amended with cellulose from Spruce 2	(Olson, Aerts et al. 2012)
GSM748501	Heterobasidion irregulare fruiting bodies collected in California 1	(Olson, Aerts et al. 2012)
GSM748502	Heterobasidion irregulare fruiting bodies collected in California 2	(Olson, Aerts et al. 2012)
GSM748503	Heterobasidion irregulare fruiting bodies collected in California 3	(Olson, Aerts et al. 2012)
GSM748504	Heterobasidion irregulare fruiting bodies collected in California 4	(Olson, Aerts et al. 2012)
GSM748505	H. irregulare grown on wood shavings from pine 1	(Olson, Aerts et al. 2012)
GSM748506	H. irregulare grown on wood shavings from pine 2	(Olson, Aerts et al. 2012)
GSM748559	H. irregulare grown on wood shavings from pine 3	(Olson, Aerts et al. 2012)
GSM748560	H. irregulare grown on wood shavings from pine 4	(Olson, Aerts et al. 2012)
GSM748572	Heterobasidion irregulare grown in liquid medium amended with lignin 1	(Olson, Aerts et al. 2012)
GSM748573	Heterobasidion irregulare grown in liquid medium amended with lignin 2	(Olson, Aerts et al. 2012)
GSM979475	H. annosum grown on pulverized Scots pine bark shavings, rep1	(Raffaello, Chen et al. 2014)
GSM979476	H. annosum grown on pulverized Scots pine bark shavings, rep2	(Raffaello, Chen et al. 2014)
GSM979477	H. annosum grown on pulverized Scots pine bark shavings, rep3	(Raffaello, Chen et al. 2014)
GSM979481	H. annosum grown on liquid malt extract medium, rep1	(Raffaello, Chen et al. 2014)
GSM979482	H. annosum grown on liquid malt extract medium, rep2	(Raffaello, Chen et al. 2014)
GSM979483	H. annosum grown on liquid malt extract medium, rep3	(Raffaello, Chen et al. 2014)
GSM979490	H. annosum grown on pulverized Scots pine heartwood shavings, rep1	(Raffaello, Chen et al. 2014)
GSM979492	H. annosum grown on pulverized Scots pine heartwood shavings, rep2	(Raffaello, Chen et al. 2014)
GSM979494	H. annosum grown on pulverized Scots pine heartwood shavings, rep3	(Raffaello, Chen et al. 2014)
GSM979515	Necrotic phloem of Scots pine infected with H. annosum, rep1	(Raffaello, Chen et al. 2014)
GSM979516	Necrotic phloem of Scots pine infected with H. annosum, rep2	(Raffaello, Chen et al. 2014)
GSM979517	Necrotic phloem of Scots pine infected with H. annosum, rep3	(Raffaello, Chen et al. 2014)
GSM979530	H. annosum grown on pulverized Scots pine sapwood shavings, rep1	(Raffaello, Chen et al. 2014)

Supplementary Table S1 (*Continued)*

GSM979531	H. annosum grown on pulverized Scots pine sapwood shavings, rep2	(Raffaello, Chen et al. 2014)
GSM979532	H. annosum grown on pulverized Scots pine sapwood shavings, rep3	(Raffaello, Chen et al. 2014)
GSM979536	Necrotic xylem of Scots pine infected with H. annosum, rep1	(Raffaello, Chen et al. 2014)
GSM979537	Necrotic xylem of Scots pine infected with H. annosum, rep2	(Raffaello, Chen et al. 2014)
GSM979538	Necrotic xylem of Scots pine infected with H. annosum, rep3	(Raffaello, Chen et al. 2014)
GSM1333665	H. annosum grown on Pine-dominant mineral forest topsoil (organic layer) 1	Unpublished
GSM1333666	H. annosum grown on Pine-dominant mineral forest topsoil (organic layer) 2	Unpublished
GSM1333667	H. annosum grown on Pine-dominant mineral forest topsoil (organic layer) 3	Unpublished
GSM1333668	H. annosum grown on Pine-dominant pristine peatland forest topsoil (organic layer) 1	Unpublished
GSM1333669	H. annosum grown on Pine-dominant pristine peatland forest topsoil (organic layer) 2	Unpublished
GSM1333670	H. annosum grown on Pine-dominant pristine peatland forest topsoil (organic layer) 3	Unpublished
GSM1333671	H. annosum grown on Pine-dominant drained peatland forest topsoil (organic layer) 1	Unpublished
GSM1333672	H. annosum grown on Pine-dominant drained peatland forest topsoil (organic layer) 2	Unpublished
GSM1333673	H. annosum grown on Pine-dominant drained peatland forest topsoil (organic layer) 3	Unpublished

[1]Biological replicates are indicated by the Arabic number at the end of the description.

Supplementary Table S2: List of primers used in this study.

Gene	Sequence used to design PCR and qPCR primers	Forward primer	Reverse primer	Amplicon length	E	Reference
PR1a	NA	CGACCAGGTAGCAGCCTATG	TCTCAACAGCCTTAGCAGCC	NA	2	Qi M, et al., 2016
PR2	NA	GGGCTGTTAATTTGCAGTATCC	GGTTTATAACATCTTGGTCTGATGG	NA	2	Qi M, et al., 2016
WRKY12	NA	CTCATCAGCTAGTTCATTTGATGC	AGCTCGGTCTTTGTTCTAAAAGC	NA	2	Qi M, et al., 2016
PI1	NA	CTTCAAAGACTATGGTGAAGTTTGC	CAGACTGAGACACATCAAGTTGC	NA	2	Qi M, et al., 2016
PR3	>PR-3 ATGGAGTTTTCTGGATCACCACTGGTATTGTTTTGTTGTGTGTTTTTCCTGTTCT TAACAGGGAGCTTGGCACAAGGCATTGGTTCTATTGTGACGAGTGACTTGTTC AACGAGATGCTGAAGAATAGGAATGACGTTAGATGTCCTGCCAATGGCTTCTA CACTTATGATGCATTCATAGCTGCTGCCAATTCCTTTCCTGGTTTTGGAACTACT GGTGATGATACTGCCCGTAGGAAAGAAATTGCTGCCTTTTTCGGTCAAACTTCT CATGAAACTACTGGTGGTTCCCTGAGTGCAGAACCATTTACAGGAGGATATTG CTTTGTTCGGCAAAATGACCAGAGTGAAAGATATTATGGTAGAGGACCCATCC AATTGACAAACCGAAATAATTATGAGAAAGCTGGAACTGCAATTCAACAAGAC CTAGTTAACAACCCTGATTTAGTAGCCACAGATGCTACTATATCATTCAAAACA GCTATATGGTTTTGGATGACAGCACAGGACAACAAGCCATCTTCCCACGACGT TATCATCGGTAGTTGGACTCCGTCTGCCGCGGATCAGGCGGCGAATCGAGTAC CAGGTTACGGTGTAATTACCAACATCATTAACGGTGGAATTGAATGTGGCATG GGTCGAAATGACGCAGTGGAAGATCGAATTGGATACTACAGGAGGTATTGTG GAATGTTAAATGTTGCTCCTGGGGAAAACTTGGACTGTTACAACCAAAGGAAC TTCGCCCAGGGCTAG	TGCCTTTTTCGGTCAAACTT	TGTAAATGGTTCTGCACTCAGG	64	1.87	This study
PR4a	>PR-4a ATGGAGAGAGTAAATAATTACTATAAGTTGTGTATGGAATTGTTTATCATGAG CATGATGGTGGCAATGGCGGCGGCACAGAGCGCTACAAACGTGAGATCAACG TATCATTTATACAACCCACAGAACATTAACTGGGATTTGAGAGCAGCAAGTGC TTTCTGCACTACTTGGGATGCCGACAAGCCTCTCACGTGGCGTCAGAAATATG GCTGGACTGCTTTCTGTGATGCTGCTGGACCTCAAGGCCAAGATTCCTGTGGT AGATGCTTGAGGGTGACGAACACAGGAACAGGAACTCAAACAACAGTGAGAA TAGTAGATCAATGCAGAAATGGAGGGCTTGATTTGGATGTAAACGTCTTTAAC CAATTGGACACAAATGGAGTGGGCTATCAGCAAGGCCACCTTATTGTCAACTA TGAATTTATCAACTGCGATGACTAA	CAACCCACAGAACATTAACTGG	TTGTCGGCATCCCAAGTAGT	69	1.9	This study
PR5	>PR-5 ATGAACTTCCTCAAAAGCTTCCCCTTTTTTGCCTTCCTTTGTTTTGGCCAATACTT TGTAGCTGTTACTCATGCTGCAACTTTTGACATTATCAACCAATGCACCTACAC AGTCTGGGCCGCGGCCTCTCCAGGTGGAGGCAGGCAGCTCAACTCGGGCCAA TCTTGGAGCATTAACGTGAACCCAGGAACAGTCCAGGCTCGCATATGGGGCC GAACCAACTGCAACTTCGATGGCAGTGGCCGAGGTAATTGTGAGACTGGAGA CTGTAACGGGATGCTAGAGTGTCAAGGCTATGGTAAACCACCCAACACTTTAG CTGAATTTGCACTTAATCAGCCTAATCAGGACTTCGTCGACATCTCTCTTGTTGA TGGATTTAACATCCCCATGGAATTCAGCCCAACTAATGGCGGGTGCCGTAACC TTAGATGCGCAGCCCCTATTAACGAGCAATGCCCAGCACAGTTGAAAACACAA GGTGGATGTAACAACCCATGTACTGTGATAAAAACCAATGAATATTGTTGTAC AAATGGGCCTGGATCATGTGGGCCTACTGATTTGTCGAGATTTTTTAAGGAAA GATGCCCAGATGCTTATAGCTATCCACAGGATGATCCAACTAGTTTGTTTACTT GTCCTTCTGGTACCAATTACAGGGTTGTCTTCTGCCCTTGA	ATGCGCAGCCCCTATTAAC	TGGGTTGTTACATCCACCTTG	67	1.88	This study

Supplementary Table S2 (*Continued)*

Gene	Sequence used to design PCR and qPCR primers	Forward primer	Reverse primer	Amplicon length	E	Reference
ERF1	>ERF1 ATGGGCTCACCACAAGAGAATTGCACTACACTTGATTTAATTAGGCAACATCTTCTTGATGATAATGTTTTCATGG AACATTATTGTCCCCAACCAATTCTTTATTCTCAAAGCTCCTCCTCTTCTGAATCTTTAAACTCCATTGCTTCTGAGC TCAATAACGATAGTTTCTCCTTTGAACCAACTCTCAATTATGCCGACACAGCCCAAAGTTCCAATCTTGATATCTCA ACCTTCTTTAACAATTCAAAAACAGAGTTCGACTGCTTTGAGTTTGAGACAAAACCAAACGTGTTAGCTGCTCGTA TTAGTTCAAATTCTCCGAAGCAAACAAGCTTCAACGAACGCAAGCCTTCTCTGAATATTGCTATACCCCTGAAGCA GCAAGAGGTTGTTCAGAAAGTGGGAATTTCCAACGAGAGCAAGAAGAAGCATTACCGAGGAGTTAGGCAACGG CCGTGGGGGAAGTTCGCCGCAGAGATTCGTGACCCGAACCGAAAGGGGACTCGGGTTTGGTTAGGAACCTTTG ACACTGCCTTAGAGGCGGCTAAGGCATATGATAGGGCGGCGTTTAAGCTTAGAGGTAGCAAAGCAATAGTTAAT TTCCCTCTCGAAGTTTCAAACTTTAAGCAACAAGATAATGAGATTCAGCCTTTGATGAACTCAAACAGGAAAAGG GTGAGAGAAACAGAGAGTGAGGAGCAACTAGTTGTCGACAAGGAAATGAAAATTGAACAAGAAAGAGTCCCAA CAGCTCCGTTAACGCCGTCAAGTTGGTCGGCGATTTGGGACAGTGGAAATGGGAAGGGTATATTTGAGGTGCCG CCTCTATCACCATTATCTCCACATATGGCTTATTCTCAGCTTGTCATGATATAA	GTTAACGCCGTCAAGTTGGT	AGAGGCGGCACCTCAAATA	72	1.93	This study
Endochitinase B	> Endochitinase B ATGAGGCTTTGTAAATTCACAGCTCTTTCTTCTCTACTATTTTCTCTCCTATTGCTTTCTGCCTCGGCAGAACAATGT GGTTCGCAGGCCGGAGGTGCGCGTTGTCCCTCGGGTCTCTGCTGCAGCAAATTTGGTTGGTGTGGTAACACCAA TGACTATTGTGGCCCTGGCAATTGCCAGAGCCAGTGCCCTGGTGGTCCCACACCTACACCGCCCACCCCACCCGG TGGTGGGGACCTCGGCAGTATCATTTCAAATTCCATGTTTGATCAGATGCTTAAGCATCGCAACGATAATGCCTG CGAAGGACATGGATTCTACAGTTACAATGCCTTTATCAATGCTGCTAGGTCTTTTCCTGGCTTTGGTACCAGTGGC GATACTACTGCCCGAGGATGGGCAACAGCACCAGATGGTCCATATGCATGGGGTTATTGCTGGCTTAGAGAACG AGGTAGCCCCGGCGACTACTGTACACCAAGTGGTCAGTGGCCTTGTGCTCCTGGTCGCAAATATTTCGGACGAG GCCCCATCCAAATTTCACACAACTATAACTACGGACCTTGTGGAAGAGCCATAGGAGTGGACCTCCTAAACAATC CTGATTTAGTGGCCACAGATCCAGTTATCTCATTTAAGTCAGCTCTCTGGTTTTGGATGACCCCTCAATCACCAAA ACCTTCTTGCCACGATGTCATCATCGGAAGATGGCAACCATCGTCTGCTGACCGCGCAGCCAATCGTCTCCCTGG ATTTGGTGTCATCACGAACATCATCAACGGTGGCCTGGAATGTGGTCATGGCACTGACTCAAGGGTCCAGGATC GCGTTGGGTTTTACAGAAGGTATTGCAGTATTCTTGGAGTTAGTCCTGGTGACAATCTTGATTGCGGCAACCAGA GGTCTTTTGGAAACGGACTTTTAGTCGATACTATGTAA	GCCTTTATCAATGCTGCTAGG	ATCCTCGGGCAGTAGTATCG	67	1.88	This study
EF1a	>EF1a ATGGGTAAAGAAAAAGTTCACATCAACATTGTGGTCATTGGCCACGTCGATTCTGGAAAGTCTACCACCACTGGT CACTTGATCTACAAGCTTGGTGGTATTGACAAGCGTGTTATTGAGAGGTTCGAGAAGGAAGCCGCTGAGATGAA CAAGAGGTCATTCAAGTATGCCTGGGTGCTTGACAAGCTTAAGGCTGAACGTGAGCGGGGTATCACCATTGATA TTGCCTTGTGGAAGTTTGAGACCACCAAATACTACTGCACTGTGATTGATGCCCCCGGACACAGGGACTTTATCA AGAACATGATCACTGGTACTTCCCAGGCTGATTGTGCTGTTCTTATTATTGACTCCACCACTGGTGGTTTTGAAGC TGGTATTTCCAAGGATGGTCAGACCCGTGAACACGCATTGCTTGCTTTCACCCTTGGTGTCAAGCAAATGATTTGC TGCTGCAACAAGATGGATGCTACCACCCCCAAGTACTCCAAGGCTAGGTATGATGAAATCGTCAAGGAAGTTTCT TCCTACCTGAAGAAGGTTGGTTACAACCCTGACAAGATTCCCTTCGTCCCCATCTCTGGTTTTGAGGGAGACAATA TGATTGAGAGGTCTACCAACCTTGACTGGTACAAGGGGCCAACCCTCCTTGAGGCTCTTGACCAGATTAATGAGC CCAAGAGGCCCACAGACAAACCCCTACGTCTTCCACTTCAGGACGTTTACAAGATTGGTGGTATTGGTACCGTCC CCGTTGGTCGTGTTGAAACTGGTGTCCTCAAGCCTGGTATGGTTGTGACCTTTGGGCCTACTGGTCTGACAACTG AAGTCAAGTCTGTAGAGATGCACCACGAAGCTCTCCTGGAGGCACTCCCCGGTGACAATGTTGGGTTCAATGTTA AGAATGTTGCTGTTAAGGATCTCAAGCGTGGTTATGTTGCCTCAAATTCCAAGGATGACCCAGCCAAAGGGGCT GCCAGTTTCACCTCCCAGGTCATCATCATGAACCATCCTGGTCAGATTGGAAATGGATATGCACCAGTTCTTGACT GCCACACTTCCCACATTGCTGTCAAGTTCGCTGAGATCTTGACCAAGATTGACAGACGTTCTGGTAAGGAACTTG AGAAGGAGCCTAAGTTCTTGAAGAATGGTGATGCTGGTATGGTTAAGATGATGCCGACCAAGCCTATGGTTGTG GAGACCTTTGCCGAGTACCCACCATTGGGTCGTTTTGCTGTGAGGGACATGCGTCAGACTGTTGCTGTTGGTGTC ATCAAGAGTGTTGACAAGAAGGACCCAACTGGCGCCAAGGTCACCAAAGCTGCTCAGAAGAAGAAGTGA	CTCCTTGAGGCTCTTGACCA	ACGTAGGGGTTTGTCTGTGG	60	1.91	This study

Supplementary Table S2 (*Continued)*

Gene	Sequence used to design PCR and qPCR primers	Forward primer	Reverse primer	Amplicon length	E	Reference
Actin	>Actin ATGGCAGATGGAGAGGATATTCAGCCACTTGTCTGTGACAATGGAACAGGAATGGTCAAGGCTGG GTTTGCTGGAGATGATGCTCCACGAGCTGTATTCCCTAGTATTGTTGGCCGGCCCCGCCATACTGG TGTGATGGTGGGTATGGGTCAGAAAGATGCCTACGTGGGAGATGAAGCTCAATCAAAAAGAGGT ATTTTAACTCTTAAATACCCAATTGAGCATGGAATTGTCAGCAACTGGGATGATATGGAGAAGATC TGGCATCATACTTTCTACAATGAGCTTCGTGTTGCGCCCGAGGAGCATCCAGTCCTCCTAACTGAAG CGCCTCTTAACCCAAAGGCTAATCGTGAAAAGATGACCCAGATTATGTTTGAGACTTTTAATACCCC AGCTATGTATGTTGCTATTCAGGCTGTCCTCTCACTGTATGCCAGTGGTCGTACCACCGGTATTGTG TTGGACTCTGGTGATGGTGTCAGCCACACCGTCCCAATTTATGAGGGGTATGCCCTCCCACATGCC ATTCTCCGTCTTGACTTGGCAGGCCGTGACCTCACTGATAGTTTGATGAAGATCCTTACCGAGCGT GGTTACATGTTCACCACCTCAGCTGAGCAGGAAATTGTCAGGGACGTGAAAGAAAAGCTTGCTTA CATAGCTCTTGACTATGAACAGGAACTCGAGACTGCAAAGACCAGCTCTTCTGTAGAGAAGAACTA TGAGCTCCCAGATGGGCAGGTGATCACCATTGGTGCTGAGCGTTTCCGTTGTCCTGAGGTCCTTTT CCAACCATCAATGATTGGAATGGAAGCTGCAGGAATCCACGAGACTACATACAACTCTATCATGAA GTGTGATGTGGATATTAGAAAGGATCTTTATGGAAACATTGTGCTCAGTGGTGGCTCTACCATGTT CCCGGGTATCGCTGATAGAATGAGCAAAGAAATTACTGCGCTTGCTCCTAGCAGCATGAAGATTAA GGTGGTGGCCCCACCAGAGAGGAAATACAGTGTCTGGATTGGAGGCTCTATCTTGGCTTCCCTCA GCACCTTCCAGCAGATGTGGATTGCTAAGGCAGAGTATGACGAATCTGGTCCGTCTATTGTCCACA GAAAGTGCTTCTGA	TACCACCGGTATTGTGTTGG	TCATAAATTGGGACGGTGTG	60	1.82	This study