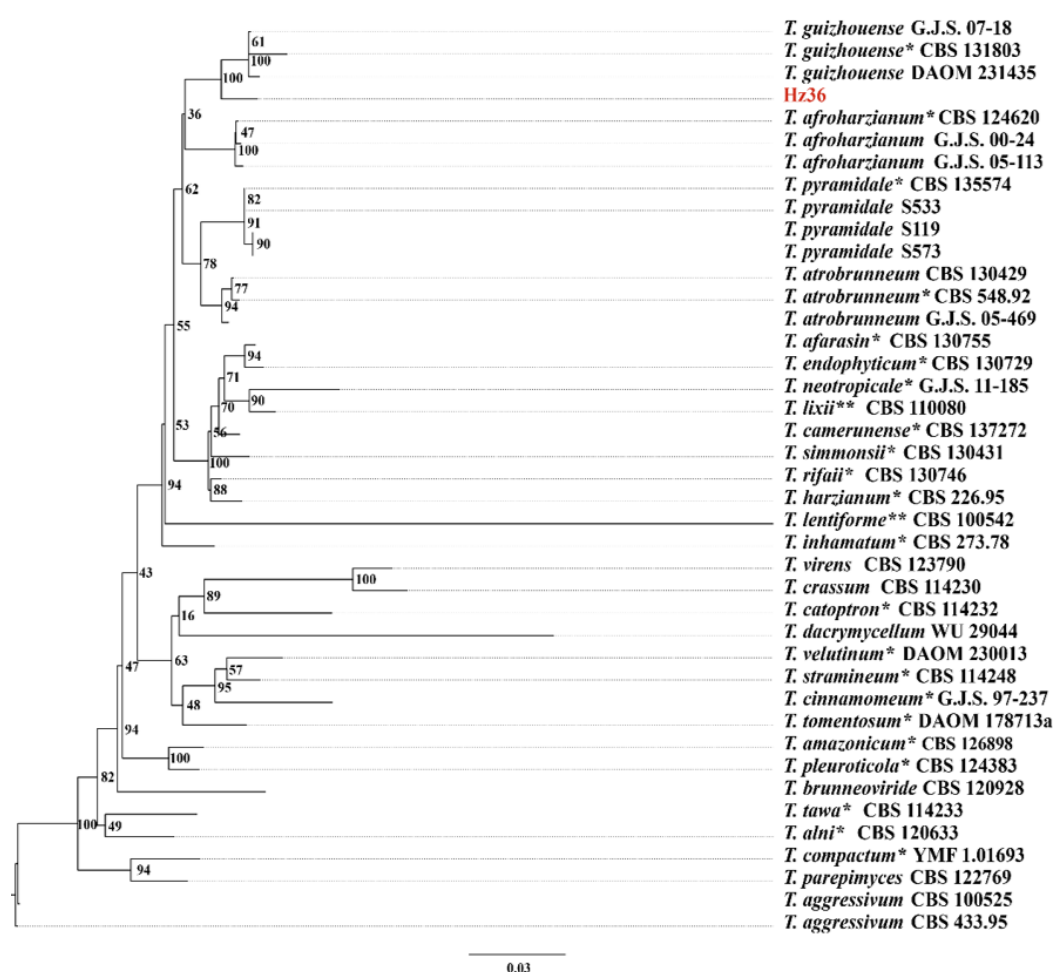


Article

Application of *Trichoderma* Hz36 and Hk37 as Biocontrol Agents against Clubroot Caused by *Plasmodiophora brassicae*

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Supplement information:



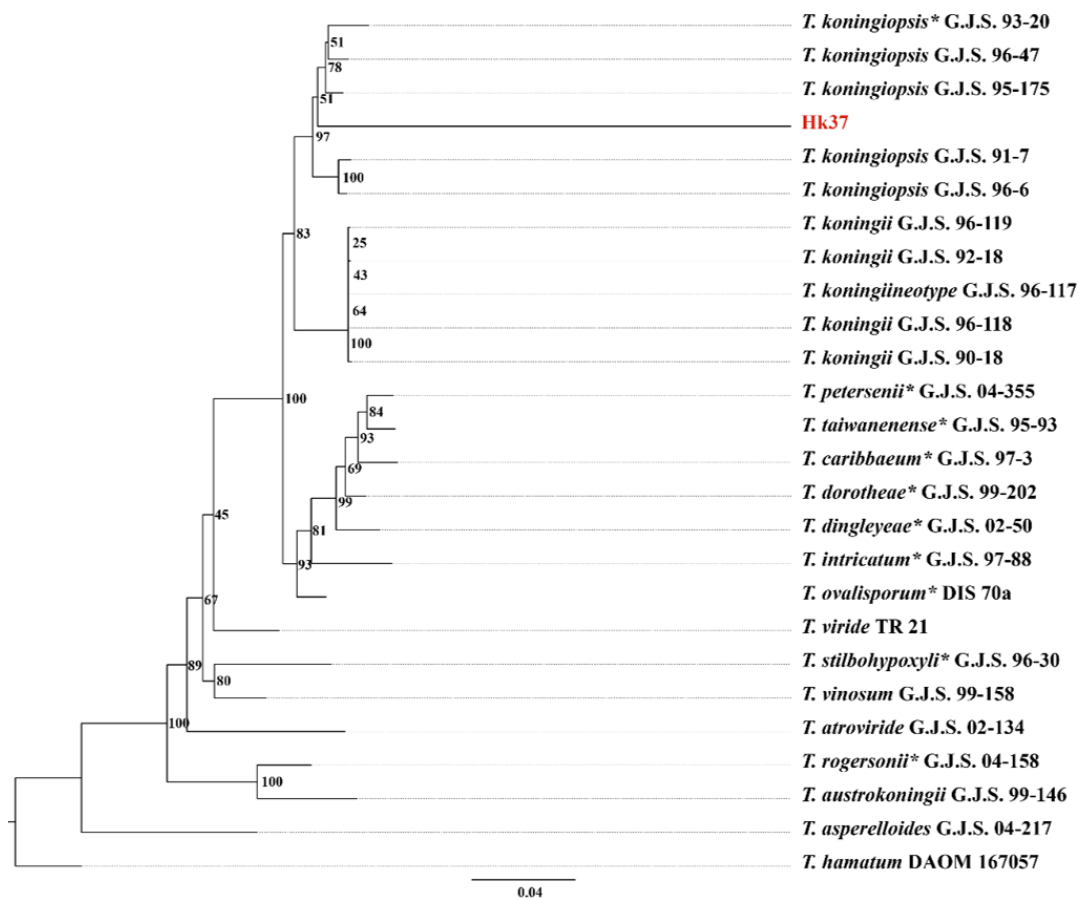


Figure S2. Identification of Hk37. Maximum likelihood tree based on (*TEF1-CAL-ACT*) sequences from 25 *Trichoderma*. *T. hamatum* DAOM 167057 was selected as the outgroup. Ex-type strains were emphasized in star. Scale bar = 0.04.

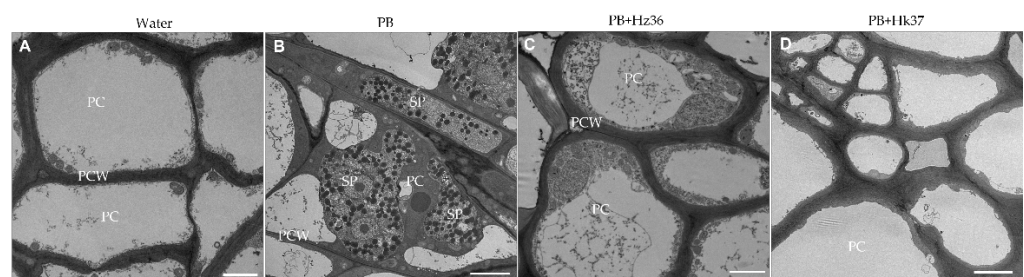


Figure S3. Transmission electron microscopy observation of *P. brassicae* treated with strain Hz36 and Hk37. (A) Rapeseed inoculated with water as a negative control. (B) Rapeseed inoculated with *P. brassicae* alone for 30 days. (C) Rapeseed co-inoculated with *P. brassicae* and Hz36 strain for 30 days. (D) Rapeseed co-inoculated with *P. brassicae* and Hk37 strain for 30 days. The scale bar = 2 μ m, PC = plant cell; PCW = plant cell wall; SP = secondary plasmodium.

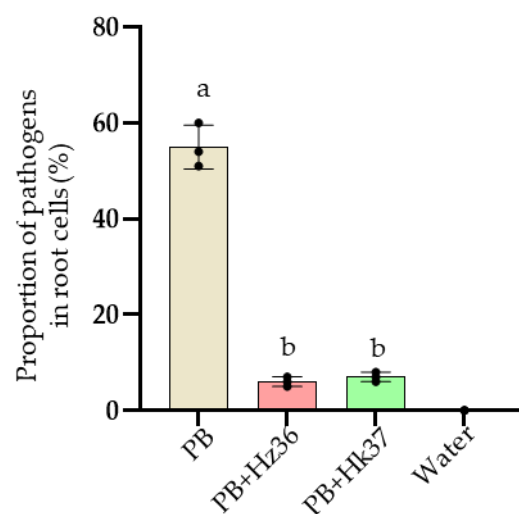


Figure S4. Proportion of *P. brassicae* in root cells of inoculated with *P. brassicae* alone or co-inoculated, inoculated with water only as a negative control. Results show the means \pm s.d. ($n = 3$ biological replicates), data analyzed with one-way ANOVA by Tukey's multiple comparisons were conducted using Prism 8. significance set at $P \leq 0.05$. Different letters (a and b) shown significantly different.

Table S1. The information of 44 endophyte fungi isolated from the symptomatic and asymptomatic roots of rapeseed infected by *P. brassicae* in severely infected fields.

Strain	domain	Phylum	Class	Order	Family	Genus	Species	Biocontrol efficiency
22	Fungi	Ascomycota	Dothideomycetes	Pleosporales	Pleosporaceae	Alternaria	Alternaria tenuissima	NA
44	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Nectriaceae	Fusarium	Fusarium proliferatum	NA
7	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	Hypocrea lixii	32.13%
38	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	Hypocrea lixii	10.92%
15	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Hypocrea	unclassified Hypocrea	NA
3	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	Mucor	Mucor circinelloides	NA
2	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	Mucor	Mucor hiemalis	NA
9	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	Mucor	Mucor hiemalis	NA
10	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	Mucor	Mucor hiemalis	NA
16	Fungi	Mucoromycota	Mucoromycetes	Mucorales	Mucoraceae	Mucor	M. racemosus	NA
39	Fungi	Oomycota	Oomycetes	Pythiales	Pythiaceae	Pythium	Pythium coloratum	NA
40	Fungi	Oomycota	Oomycetes	Pythiales	Pythiaceae	Pythium	Pythium coloratum	NA
41	Fungi	Oomycota	Oomycetes	Pythiales	Pythiaceae	Pythium	Pythiumcoloratum	NA
32	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviride	NA
34	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviride	NA
17	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
19	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
20	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
23	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
24	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
33	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
5	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
6	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
11	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
12	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
13	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
14	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	19.39%
25	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
26	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
27	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	-10.28%
28	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
29	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
43	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma atroviridis	NA
36	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma guizhouense	52.18%
1	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma harzianum	NA
8	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma harzianum	NA
21	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma harzianum	NA
42	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma harzianum	10%
37	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma koningiopsis	68.01%
4	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma viride	NA
18	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma viride	5%
30	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma viride	NA
31	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma viride	NA
35	Fungi	Ascomycota	Sordariomycetes	Hypocreales	Hypocreaceae	Trichoderma	Trichoderma viride	NA

Note: Fonts marked in red indicated the strain was selected against *P. brassicae* in Arabidopsis in the green house condition, inoculated with *P. brassicae* for 21 days.

Table S2. Strains used in Hz36 phylogenetic analysis and their GenBank numbers.

Species	Culture number	GenBank accession number			
		<i>ACT</i>	<i>TEF1</i>	<i>CAL</i>	<i>ITS</i>
<i>Trichoderma afarasin</i> *	CBS 130755	FJ442536	AF348093	FJ442388	AY027784
<i>Trichoderma afroharzianum</i> *	CBS 124620	-	FJ463301	FJ442370	FJ442265
<i>Trichoderma afroharzianum</i>	G.J.S. 00-24	FJ442488	AF443940	AF442880	AF443922
<i>Trichoderma afroharzianum</i>	G.J.S. 05-113	FJ442571	FJ463378	FJ442371	FJ442235
<i>Trichoderma aggressivum</i>	CBS 100525	FJ442433	AF348095	AF442859	AF057600
<i>Trichoderma aggressivum</i>	CBS 433.95	FJ442436	AF348097	FJ442279	FJ442605
<i>Trichoderma alni</i> *	CBS 120633	GQ250315	EU498312	EU498326	EU518651
<i>Trichoderma amazonicum</i> *	CBS 126898	-	HM142376	-	HM142358
<i>Trichoderma atrobrunneum</i> *	CBS 548.92	FJ442528	AF443942	AF442883	AF443924
<i>Trichoderma atrobrunneum</i>	G.J.S. 05-469	FJ442502	FJ463304	FJ442340	FJ442629
<i>Trichoderma atrobrunneum</i>	CBS 130429	FJ442525	AF443943	AF442886	AF443926
<i>Trichoderma brunneoviride</i>	CBS 120928	-	EU498318	EU498330	EU518661
<i>Trichoderma camerunense</i> *	CBS 137272	FJ442537	AF348107	AF442875	AY027780
<i>Trichoderma catoptron</i> *	CBS 114232	FJ442584	AY737726	FJ442387	AY737766
<i>Trichoderma cinnamomeum</i>	G.J.S. 97-237	FJ442582	AY737732	JN133524	AY737759
<i>Trichoderma compactum</i> *	YMF 1.01693	-	AY941824	-	-
<i>Trichoderma crassum</i>	CBS 114230	-	JN133572	-	-
<i>Trichoderma dacrymycellum</i>	WU 29044	-	FJ860633	-	FJ860749
<i>Trichoderma endophyticum</i> *	CBS 130729	FJ442445	FJ463319	FJ442292	FJ442243
<i>Trichoderma guizhouense</i> *	CBS 131803	-	JN215484	-	JN191311
<i>Trichoderma guizhouense</i>	DAOM 231435	-	EF191321	FJ577721	EF191296
<i>Trichoderma guizhouense</i>	G.J.S. 07-18	FJ442521	FJ463390	FJ442355	FJ442641
<i>Trichoderma harzianum</i> *	CBS 226.95	FJ442567	AF348101	AF442864	AJ222720
<i>Trichoderma inhamatum</i> *	CBS 273.78	FJ442561	AF348099	AF442891	FJ442680
<i>Trichoderma lentiforme</i> **	CBS 100542	AF469193	AF469195	AF469191	AF469189
<i>Trichoderma lixii</i> **	CBS 110080	FJ442533	AF443938	AF442872	AF443920
<i>Trichoderma neotropicale</i> *	G.J.S. 11-185	KP115268	HQ022771	KP115279	HQ022407
<i>Trichoderma parepimyces</i>	CBS 122769	-	FJ860664	-	FJ860800
<i>Trichoderma pleuroticola</i> *	CBS 124383	JN133598	HM142381	JN133538	HM142362
<i>Trichoderma pyramidale</i> *	CBS 135574	-	KJ665699	-	-
<i>Trichoderma pyramidale</i>	S119	-	KJ665696	-	-
<i>Trichoderma pyramidale</i>	S533	-	KJ665697	-	-
<i>Trichoderma pyramidale</i>	S573	-	KJ665698	-	-
<i>Trichoderma rifaii</i> *	CBS 130746	FJ442474	FJ463324	-	FJ442663
<i>Trichoderma simmonsii</i> *	CBS 130431	FJ442526	AF443935	AF442869	AF443917
<i>Trichoderma stramineum</i> *	CBS 114248	FJ442583	AY737746	FJ442386	AY737765
<i>Trichoderma tawa</i> *	CBS 114233	FJ442570	FJ463313	FJ442406	AY737756
<i>Trichoderma tomentosum</i> *	DAOM 178713a	JN133612	AY750882	JN133548	EU330958
<i>Trichoderma velutinum</i> *	DAOM 230013	JN133614	AY937415	JN133550	AF149873
<i>Trichoderma virens</i>	CBS 123790	FJ442589	AY750894	FJ442404	DQ083023

Note: Ex-type strains were emphasized in star.

Table S3. Strains used in Hk37 phylogenetic analysis and their GenBank numbers.

Species	Strain	GenBank accession number		
		<i>TEF1</i>	<i>CAL</i>	<i>ACT</i>
<i>Trichoderma atroviride</i>	G.J.S. 02-134	DQ307547	-	-
<i>Trichoderma austrokonigii</i> *	G.J.S. 99-146	DQ307561	DQ379001	DQ367716
<i>Trichoderma asperellum</i>	G.J.S. 04-217	DQ381958	DQ379002	DQ333564
<i>Trichoderma caribbaeum</i> *	G.J.S. 97-3	DQ284977	DQ367688	DQ328607
<i>Trichoderma dingleyae</i> *	G.J.S. 02-50	DQ284978	DQ370461	DQ367718
<i>Trichoderma dorotheae</i> *	G.J.S. 99-202	DQ307536	DQ367706	DQ323454
<i>Trichoderma hamatum</i>	DAOM 167057	AF456911	DQ122154	DQ111962
<i>Trichoderma intricatum</i> *	G.J.S. 97-88	AY376060	DQ367680	AY376684
<i>Trichoderma konigii</i>	G.J.S. 96-117	AF456909	DQ367686	DQ341180
<i>Trichoderma konigii</i>	G.J.S. 90-18	DQ289007	DQ370463	DQ341181
<i>Trichoderma konigii</i>	G.J.S. 92-18	DQ289005	DQ370458	DQ367712
<i>Trichoderma konigii</i>	G.J.S. 96-118	DQ288996	DQ367683	DQ341178
<i>Trichoderma konigii</i>	G.J.S. 96-119	DQ289003	DQ367681	DQ341179
<i>Trichoderma koningiopsis</i>	G.J.S. 91-6	DQ307539	DQ367705	DQ323446
<i>Trichoderma koningiopsis</i>	G.J.S. 91-7	DQ284969	DQ367689	DQ323449
<i>Trichoderma koningiopsis</i>	G.J.S. 95-175	AF456910	DQ367707	DQ323448
<i>Trichoderma koningiopsis</i>	G.J.S. 96-47	AY376044	DQ367670	AY376677
<i>Trichoderma koningiopsis</i> *	G.J.S. 93-20	DQ284966	DQ367694	DQ381954
<i>Trichoderma ovalisporum</i> *	DIS 70a	AY376037	DQ379005	AY376671
<i>Trichoderma petersenii</i> *	G.J.S. 04-355	DQ284980	DQ367691	DQ333570
<i>Trichoderma rogersonii</i> *	G.J.S. 04-158	DQ307563	DQ370460	DQ333567
<i>Trichoderma stilbohypoxyli</i> *	G.J.S. 96-30	DQ109546	DQ122161	DQ111967
<i>Trichoderma taiwanense</i> *	G.J.S. 95-93	DQ284973	DQ367685	DQ323455
<i>Trichoderma viride</i>	TR 21	AY376054	DQ122165	AY376681
<i>Hypocrea vinosa</i>	G.J.S. 99-158	AY376047	DQ122158	AY376680

Note: Ex-type strains were emphasized in star.

Table S4. Primer sequences used in this paper.

Primer name	Primers sequence (5'→3')	Target
ITS1	TCCGTAGGTGAACCTGCGG	ITS
ITS4	TCCTCCGCTTATTGATATGC	ITS
ITS5	GGAAGTAAAAGTCGTAACAAGG	ITS
Tact1	TGGCACCACACCTTCTACAATGA	<i>ACT</i>
Tact2	TCTCCTTCTGCATACGGTCGGA	<i>ACT</i>
EF1-728F	CATCGAGAAGTTCGAGAAGG	<i>TEF1</i>
EF1-1R	GCCATCCTTGGGAGATACCAGC	<i>TEF2</i>
CAL-228F	GAGTTCAAGGAGGCCTTCTCCC	<i>CAL</i>
CAL-737R	CATCTTTCTGGCCATCATGG	<i>CAL</i>
Pbactin-F	CACCGACTACCTGATGAA	qRT-PCR
Pbactin-R	CAGCTTCTCCTTGATGTC	qRT-PCR
Atactin-F	AATCCACGAGACAACCTA	qRT-PCR
Atactin-R	AGCGATACCTGAGAACATA	qRT-PCR
Bnactin-F	TGAAGATCAAGGTGGTCGCA	qRT-PCR
Bnactin-R	GAAGGCAGAAACACTTAGAAG	qRT-PCR