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Supplementary Material

***Brachypodium distachyon* genotypes vary in resistance to *Rhizoctonia solani* AG8**

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Table S1. Information on tagged genes of T-DNA lines included in *R. solani* AG8 disease screening experiments

Lines were chosen, from those available, based on literature evidence that disruption of putative T-DNA tagged genes could have an effect on disease resistance, root growth or interaction with cell wall degrading enzymes produced by *R. solani* AG8.

JJ line number	T-DNA tagged gene	Construct ^A ; T-DNA location	NCBI Protein-BLAST sequence homology with predicted or known genes, <i>E</i> -value	Notes on gene family homologues from cited references and The UniProt Consortium (2012)
77	<i>Bradi3g36010</i>	pOL001; in gene	Predicted: nucleobase-ascorbate transporter 6-like [<i>B. distachyon</i>], 0.0	NAT6 expressed in Arabidopsis seedling roots and lateral root primordia (UniPROT Q27GI3) (Maurino <i>et al.</i> , 2006).
654	<i>Bradi1g23450</i>	pOL001; in gene	Predicted: polyol transporter 5-like [<i>B. distachyon</i>], 0.0	Sugar-proton symporter; highly expressed in Arabidopsis roots, induced by wounding and insect feeding (UniPROT Q8VZ80) (Klepek <i>et al.</i> , 2005; Reinders <i>et al.</i> , 2005).
705	<i>Bradi2g48120</i>	pOL001; in gene	Predicted: ras-related protein Rab7-like [<i>B. distachyon</i>], 3e-150	Small GTP-binding proteins; wheat Rab7 is involved in response to stripe rust and abiotic stress (Liu <i>et al.</i> , 2012).
2426	<i>Bradi3g29780</i>	pJJ2LBA activation tagging; ~ 250 bases downstream	Predicted: probable aquaporin TIP3-1-like [<i>B. distachyon</i>], 0.0	Facilitated water movement and possible root water uptake (Sakurai <i>et al.</i> (2005). Aligns with <i>OsTIP3;1</i> , expressed in leaves and at lower levels in roots; 100% coverage, 87% identity, 2e-150, (UniPROT Q9FWV6).
2596	<i>Bradi3g04080</i>	pJJ2LB; ~240 bases upstream	Predicted: endoglucanase 5-like [<i>B. distachyon</i>], 0.0	Cell wall synthesis (Liberti <i>et al.</i> 2004). Glycosyl hydrolase 9 family; secreted protein hydrolyses linkages in cellulose, lichenin and cereal beta-D-glucans (UniPROT Q9M995).
2771	<i>Bradi3g54950</i>	pJJ2LB; in gene	Predicted: dihydroflavonol-4-reductase-like [<i>B. distachyon</i>], 0.0	Involved in flavonoid and pigment biosynthesis (UniPROT P51102); DFR involved in response to fungal and bacterial pathogens in rice (Hayashi <i>et al.</i> , 2005).
2892	<i>Bradi3g33510</i>	pJJ2LB; in gene	Predicted: protein ARABIDILLO 1-like [<i>B. distachyon</i>], 0.0	Promoter of lateral root development in Arabidopsis (Coates <i>et al.</i> , 2006).
3175	<i>Bradi3g39050</i>	pJJ2LB; in gene	Predicted: DNA (cytosine-5)-methyltransferase 3-like [<i>B. distachyon</i>], 0.0	May be involved in DNA methylation and gene silencing (UniPROT Q8LPU5).

3400	<i>Bradi2g47210</i>	pJJ2LB; intergenic ~4.2 kb downstream	Predicted: basic endochitinase A-like isoform 1 [<i>B. distachyon</i>], 0.0	Glycosyl hydrolase 19 family; hydrolysis of linkages in chitin and chitodextrins (UniPROT P29022). Defense against chitin-containing fungal pathogens (Huynh <i>et al.</i> , 1992).
3794	<i>Bradi1g54290</i>	pJJ2LB; ~ 790 bases upstream	Late embryogenesis abundant hydroxyproline-rich glycoprotein [<i>Arabidopsis thaliana</i>], 2e-28	LEAs are involved in desiccation survival, abiotic stress tolerance (Hundertmark and Hinch 2008), root architecture and necrotrophic disease response (Salleh <i>et al.</i> , 2012).
4243	<i>Bradi5g27660</i>	pJJ2LB; ~ 590 bases upstream	Predicted: U-box domain-containing protein 25-like [<i>Fragaria vesca</i> subsp. <i>vesca</i>], 5e-54	Functions as an E3 ubiquitin ligase (UniPROT Q9LT79). Involved in defence response; response to chitin (Libault <i>et al.</i> , 2007).
4774	<i>Bradi3g39700</i>	pJJ2LB; ~ 350 bases upstream	Predicted: wall-associated receptor kinase 5-like [<i>B. distachyon</i>], 0.0	Serine/threonine protein kinase; induced by salicylic acid and pathogens (He <i>et al.</i> 1999) (UniPROT Q9LMN7).
5088	<i>Bradi3g14370</i>	pJJ2LBP2 gene trap; in gene	Predicted: probable beta-1,3-galactosyltransferase 20-like [<i>B. distachyon</i>], 0.0	Glycosyltransferase family 31, galectin domain; protein modification and glycosylation; N-glycan synthesis; expressed mainly in stems (UniPROT A7XDQ9) (Strasser <i>et al.</i> , 2007). Galectins are involved in mammalian defence response (Sato <i>et al.</i> , 2009).
7557	<i>Bradi3g16430</i>	pJJ2LBP2 gene trap; in gene	Predicted: probable LRR receptor-like serine/threonine-protein kinase At3g47570-like [<i>B. distachyon</i>], 0.0	LRRs often involved in plant defence response (Marone <i>et al.</i> , 2013) (UniPROT COLGP4).
8634	<i>Bradi2g50980</i>	pJJ2LBA activation tagging; in gene	Glycosyl hydrolase, family 43 protein [<i>Zea mays</i>], 0.0	Glycosyl hydrolase family 43 involved in plant cell wall modification (Gilbert <i>et al.</i> , 2008) (UniPROT B6U6P8).
8913	<i>Bradi1g69610</i>	pJJ2LBA activation tagging; ~ 580 bases upstream	Predicted: glucan endo-1,3-beta-glucosidase 3-like [<i>B. distachyon</i>], 0.0	Glycosyl hydrolase family 17; involved in wheat abiotic and biotic defence response (UniPROT P52409).
9212	<i>Bradi1g62970</i>	pJJ2LBA activation tagging; in gene	RING-H2 finger protein ATL5 [<i>Triticum urartu</i>], 5e-27	Aligns with a capsicum RING-H2 E3 ubiquitin ligase protein involved in biotroph defence response, 90% query coverage, 32% amino acid identity, 4e-17 (UniPROT G0T3B3) (Lee <i>et al.</i> , 2011).

9278	<i>Bradi1g68540</i>	pJJ2LBA activation tagging; in gene	Predicted: translocase of chloroplast 34, chloroplastic-like [<i>B. distachyon</i>], 0.0	GTPase, imports protein precursors into chloroplast, light-induced, expressed mostly in roots and flowers (UniPROT Q38906).
9840	<i>Bradi1g23640</i>	pJJ2LBA activation tagging; in gene	Predicted: glucan endo-1,3-beta-glucosidase 4-like [<i>B. distachyon</i>], 0.0	Glycosyl hydrolase family 17; inferred carbohydrate metabolism and defence response (UniPROT Q94CD8).

^A Construct details are given in Bragg *et al.* (2012)

Table S2. Description of methods used for screening and confirmation activities with natural accession and T-DNA collections. Soil A, ‘Barley mix’; soil B, 50% sand:‘Special’

Analysis method ‘Rs/C’, described in the main text method section, uses the ratio of *R. solani* AG8-inoculated (Rs) to uninoculated control (C) root length to compare disease resistance between lines. Analysis method ‘Rs only’ was tested as a means of reducing the number of plants required per experiment. Disease resistance comparison is based on the ratio of untransformed root length measurements of the T-DNA line to the reference line (Bd 21-3) in *R. solani* AG8-inoculated treatments. *Average seed weight* and *Days to emergence* were included as fixed terms in the REML analysis to partially account for endogenous plant vigour in control plants. Application of this method to initial screening data also analysed with method ‘Rs/C’ gave a positive correlation for resistance rankings ($R^2 = 0.5599$, $P = 0.005$), but in practice method ‘Rs only’ was less robust than method ‘Rs/C’.

Collection	Expt	Activity	Number of lines	Reps ^A	Soil	Days to harvest	Analysis method
Accessions	1	Screening	26	3	A	22	Rs/C
Accessions	2	Confirmation	4	2	B	26	Rs/C
T-DNA	1	Screening	11	3 ^B	A	22	Rs/C
T-DNA	2	Screening	9	1	A	10, 18, 26	Rs only
T-DNA	3	Screening Screen./Conf.	10 3 ^C	1	B	26	Rs only Rs/C
T-DNA	4	Confirmation	7	1	B	26	Rs/C

^A Replicate experiments sown at different times.

^B Except lines 654-9 and 3400-2

^C Control treatments were included for three of the 10 lines. This was a confirmation activity for line 77-5.

Table S3. Phenotype measurements for natural accessions in screening and confirmation experiments with *R. solani* AG8-inoculated (Rs) and control (C) treatments

Means are followed by the ratio of inoculated to control treatment values.

Line	Total root length ($\sqrt{\text{cm}}$)			Leaf number		
	Rs	C	Ratio	Rs	C	Ratio
<i>Screening activity</i>						
BdTR 13a	7.47 ^A	15.7 ^A	0.48	3.52	3.94 ^A	0.89
BdTR 1i	8.54	16.8 ^A	0.51	3.28	3.69	0.89
Adi-12	8.69	16.6 ^A	0.52	3.43	3.64	0.94
Koz-1	8.38	15.3 ^A	0.55	3.30	3.61	0.91
Bd 21-3	8.70 ^A	15.3 ^A	0.57	3.26 ^A	3.55	0.92
BdTR 10c	8.61	14.8	0.58	3.25 ^A	3.59	0.90
BdTR 5i	9.64	16.7 ^A	0.58	3.08 ^A	3.33 ^A	0.93
BdTR 11i	8.72	14.9	0.59	3.15 ^A	3.53	0.89
Bd 21	9.46	15.9 ^A	0.59	3.25 ^A	3.45	0.94
BdTR 13c	8.23 ^A	13.6	0.61	3.39	3.73 ^A	0.91
BdTR 10o	10.1	16.1 ^A	0.63	3.36	3.67	0.91

BdTR 2g	10.2	16.3 ^A	0.63	3.51	3.74 ^A	0.94
Adi-10	10.2	15.8 ^A	0.64	3.51	3.74 ^A	0.94
BdTR 3c	9.58	15.0	0.64	3.38	3.52	0.96
Bd 3-1	9.63	14.7	0.65	3.56	3.71	0.96
Tek-4	8.50	12.8	0.66 ^B	3.11 ^A	3.20 ^A	0.97
Cas 2	9.76	14.4	0.68	3.16 ^A	3.38	0.94
Abr 2	8.70	12.7	0.69 ^B	2.92 ^A	3.13 ^A	0.93
Bd 1-1	8.45 ^A	12.3	0.69 ^B	3.12 ^A	3.33 ^A	0.94
BdTR 9k	11.0	15.8 ^A	0.69	3.35	3.53	0.95
Bd 2-3	10.8	15.3 ^A	0.70	3.32	3.52	0.94
Bd 18-1	10.4	14.5	0.71 ^B	3.63	3.84 ^A	0.94
Arn 1	10.2	14.4	0.71 ^B	3.48	3.66	0.95
BdTR 12c	10.7	14.5	0.74 ^B	3.59	3.49	1.03 ^B
Bd 30-1	7.68 ^A	9.91 ^A	0.77 ^B	2.82 ^A	3.11 ^A	0.90
Koz-3	10.4	13.3	0.78 ^B	3.47	3.53	0.98 ^B

Confirmation activity

BdTR 13a	9.2 ^A	21	0.44 ^C	3.9 ^A	4.5	0.88 ^C
Bd 30-1	11 ^A	20	0.53 ^C	4.1	4.5	0.91
Adi-10	14	21	0.68	4.0	4.2	0.95

Koz-3 14 19 0.72 4.1 4.3 0.94

^A Significantly different from the same Koz-3 treatment in the same activity (5% LSD).

^B Line x Inoculum interaction significantly different from Bd 21-3 ($P < 0.05$) in the screening activity. Line Bd 21-3 is used in this comparison, as it was included in every experiment.

^C Line x Inoculum interaction significantly different from Koz-3 ($P < 0.05$) in the confirmation activity.

Table S4. Comparison of phenotype measurements for T-DNA lines and the reference line Bd 21-3 in screening and confirmation experiments in *R. solani* AG8-inoculated (Rs) and control (C) treatments

Means are followed by the ratio of inoculated to control treatment values. ‘Rs only’ root length ratio calculation is described in Table S2.

JJ line number	Total root length ($\sqrt{\text{cm}}$)			Leaf number			‘Rs only’ root length ratio
	Rs	C	Ratio	Rs	C	Ratio	
<i>First screening activity</i>							
Bd 21-3	6.8	13	0.52	3.0	3.2	0.94	0.93
77-5	8.5	12	0.70 ^B	3.3	3.4	0.98	1.82 ^B
654-9	6.0	11	0.56	3.0	3.3	0.93	0.87
705-4	8.3	13	0.65	3.3	3.4	0.97	1.08
2426-11	8.0	14	0.57	3.2	3.3	0.96	0.93
2771-5	7.0	13	0.55	3.1	3.3	0.94	0.90
2892-10	6.1	13	0.48	3.0	3.3	0.91	0.98
3175-12	6.6	13	0.49	3.1	3.5	0.88	0.92
3400-2	6.3	14	0.46	3.5	3.6	0.98	0.42
3794-5	8.0	12	0.67 ^B	3.2	3.2	0.99	1.72

4774-6	5.5	10	0.56	2.9	3.3	0.90	1.15
5088-4	7.3	11 ^A	0.68 ^B	3.1	3.2	0.98	1.86

Second screening activity^C

Bd 21-3	10	14	0.70	3.9	3.9	0.98	1.04
2596-2							1.23
2771-4							1.13
4243-11							0.85
7557-2							1.07
8634-6							1.42
8913-5							1.10
9212-15							1.65 ^B
9278-8							1.49
9840-9							1.21

Table S4 cont.

JJ line number	Total root length ($\sqrt{\text{cm}}$)			Leaf number			'Rs only' root length ratio
	Rs	C	Ratio	Rs	C	Ratio	
<i>Third screening activity</i>							
Bd 21-3	9.5	17	0.56	3.5	3.9	0.92	0.87
77-5	11	19	0.57	3.9	4.2	0.93	1.10
2426-8	13	20	0.64	4.1	4.2	0.96	2.25
2426-11							2.25
2596-2							2.32
2596-9							1.12
2596-10							2.18
3794-2							1.39
3794-5							1.10
9212-8							0.83
9212-15	9.2	20	0.45	3.7	4.1	0.90	1.45
<i>Confirmation activity</i>							
Bd 21-3	11	20	0.56	3.5	3.9	0.92	
2596-2	14	20	0.67	3.8 ^A	4.0	0.96	
2596-9	13	20	0.65	3.8 ^A	4.0	0.96	

3794-2	11	21	0.51	3.7 ^A	4.0 ^A	0.92
5088-2	12	18	0.66	3.8 ^A	3.9	0.97
5088-4	12	17 ^A	0.70 ^B	3.7	3.8	0.98
5088-5	13	20	0.66	3.8 ^A	3.9	0.96
9212-15	11	22	0.48	3.7 ^A	4.1 ^A	0.91

^A Significantly different from Bd 21-3 in the same activity (5% least significant difference).

^B Line x Inoculum interaction significantly different from Bd 21-3 ($P < 0.05$) in the same activity.

^C Values for the harvest at 26 days after sowing.

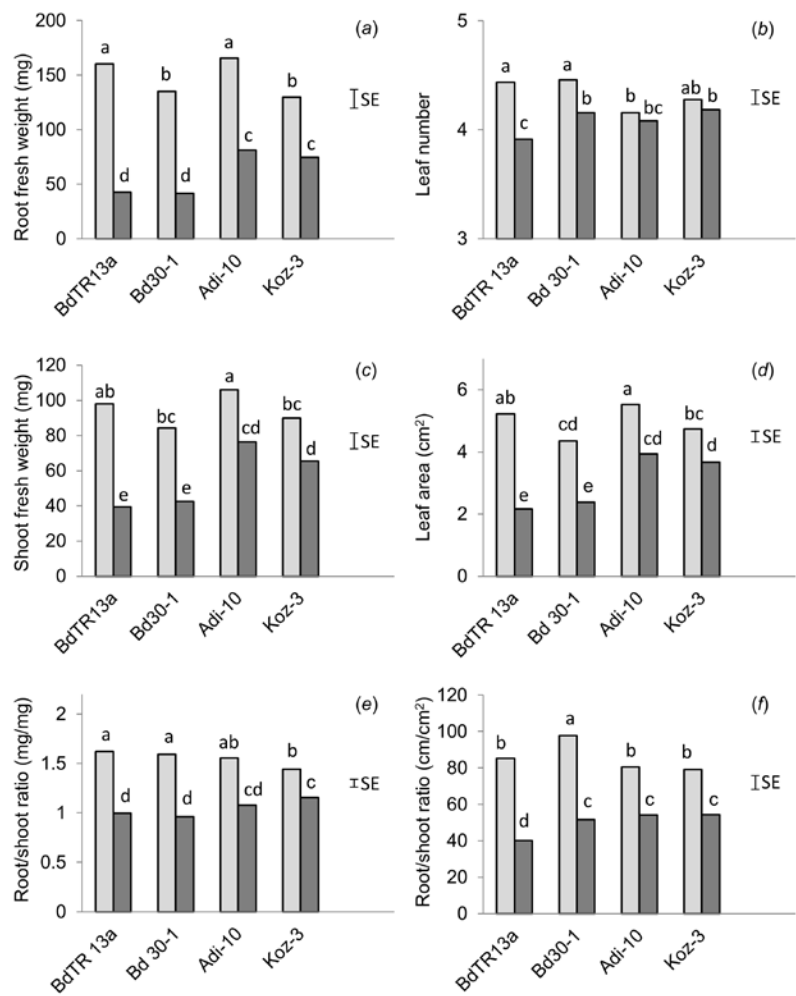


Figure S1. Natural accession screening activity. Predicted means for a) total root length, b) leaf number, c) leaf 1 length, and d) leaf 2 length, plotted against average seed mass for 26 natural accessions grown in control (white circles) and *R. solani* AG8-inoculated soil (grey circles).

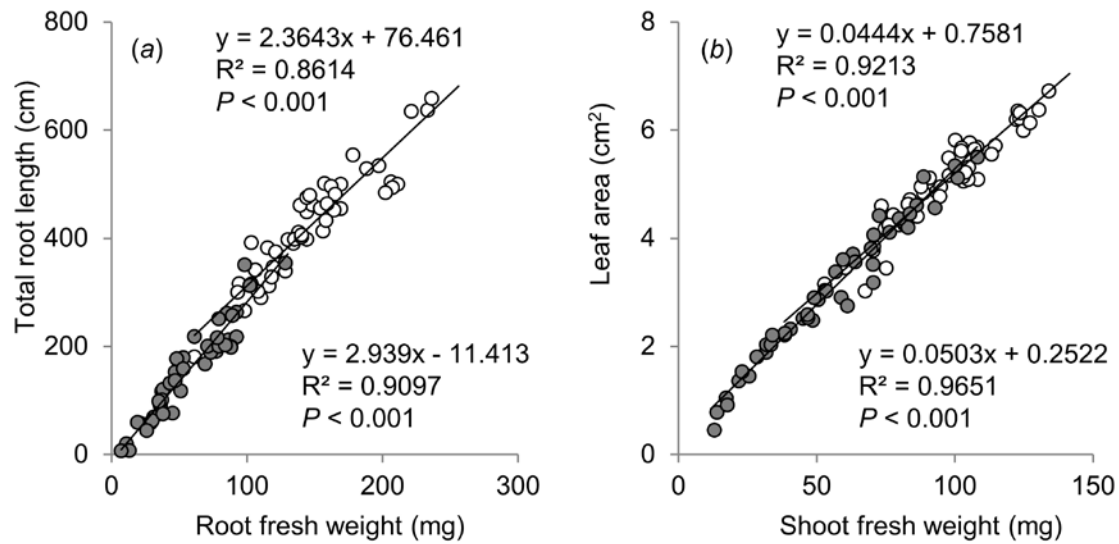


Figure S2. Natural accession confirmation activity. Correlation between a) root fresh weight and root length, and b) shoot fresh weight and leaf area, for individual plants of four accessions grown in control (white circles) and *R. solani* AG8-inoculated (grey circles) treatments.

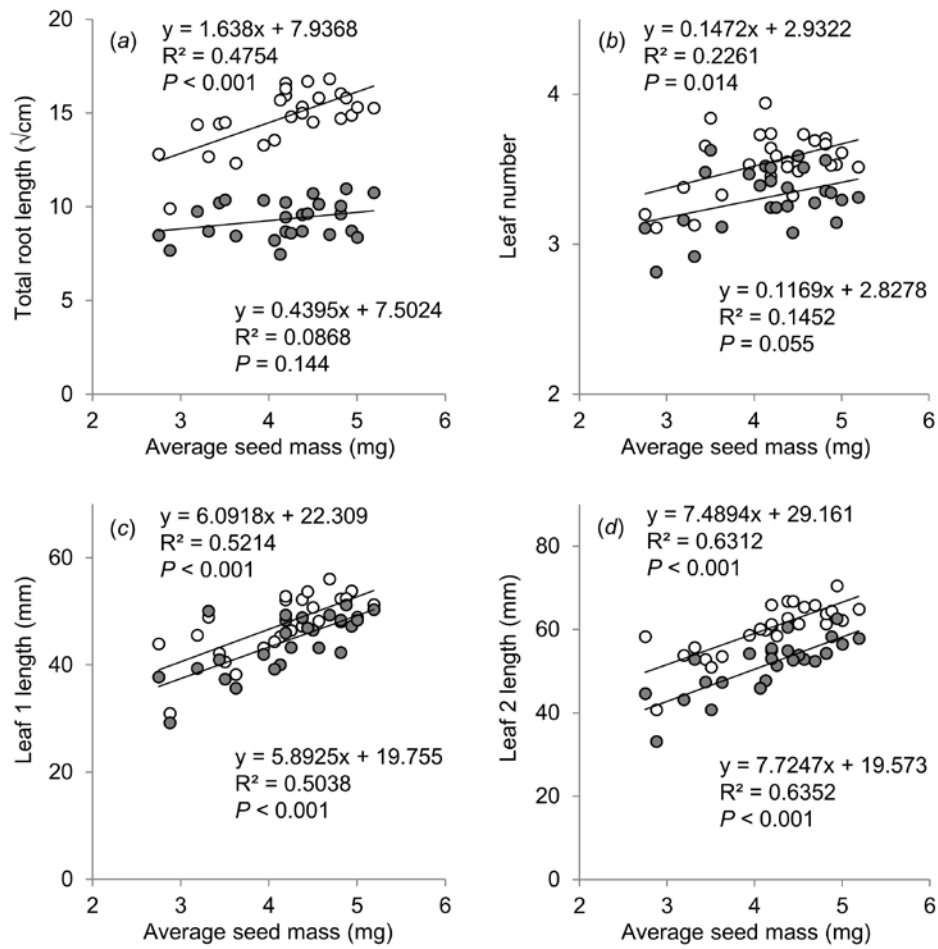


Figure S3. Natural accession screening activity. Predicted means for a) total root length, b) leaf number, c) leaf 1 length, and d) leaf 2 length, plotted against average seed mass for 26 natural accessions grown in control (white circles) and *R. solani* AG8-inoculated soil (grey circles).

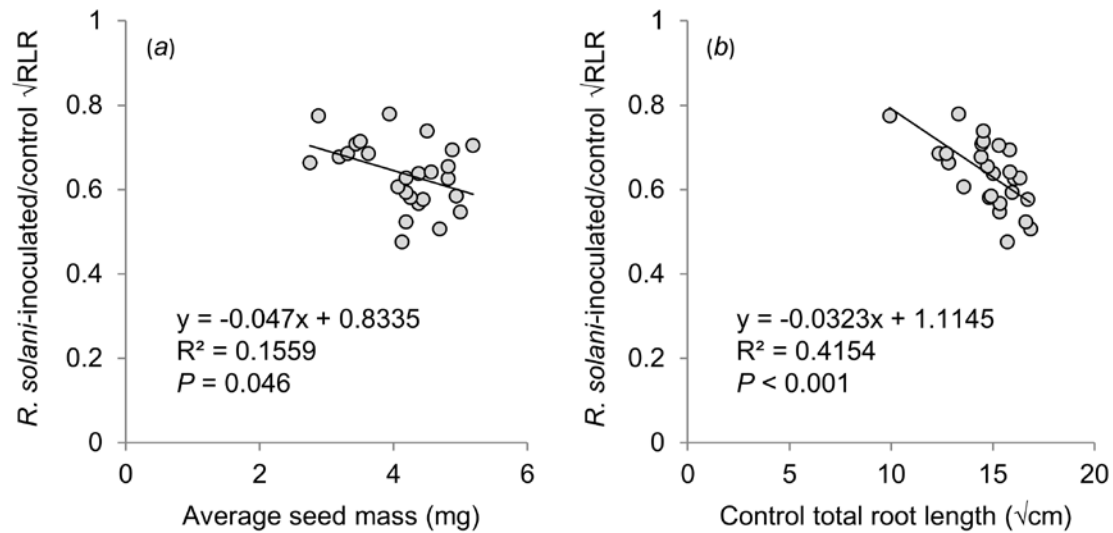


Figure S4. Correlation in the natural accession screening experiments between transformed *R. solani* AG8/control root length ratio and a) average seed mass, and b) root length in the control treatment.

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