Table S1. Analysis of F_2 progeny of a cross *hvexpb5.i* x parent cv. 'Sebastian' (WT). The *hvexpb5.i* mutant carries C773T mutation in the *HvEXPB5* gene and exhibits extremely short root hairs.

Genotypic classes in regards to C773T mutation in the *HvEXPB5* gene	Number of F_2 plants with	
	Wild type root hairs	Short root hairs
Homozygous for mutation	13	20
Heterozygous for mutation	75	27
Homozygous for the WT allele	39	0

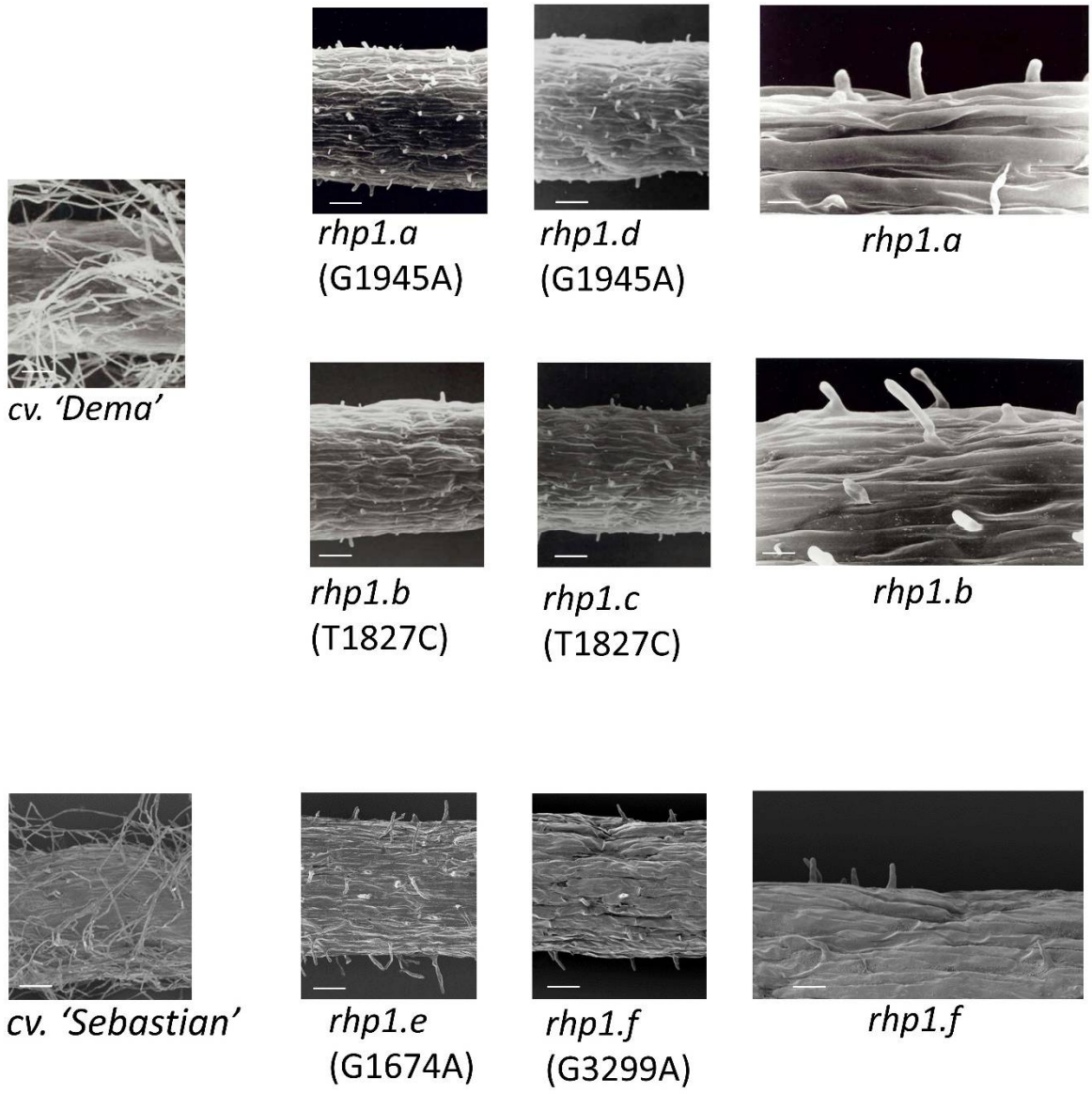


Figure S1. SEM images of barley *rhp* (*root hair primordia*) mutants and their parent varieties. Root hair zone of 5-7-old day old seedlings. Bars = 100 μm.

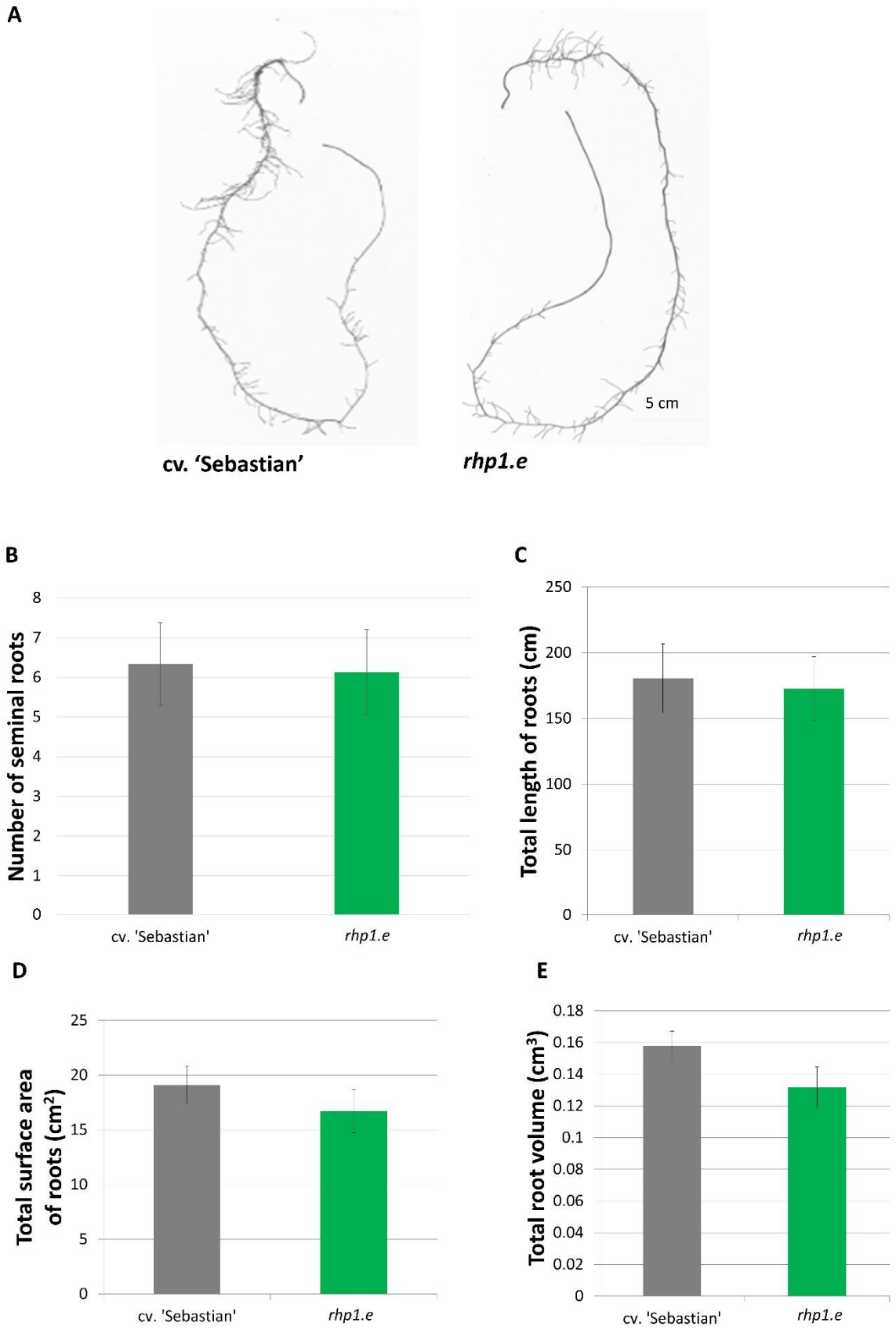


Figure S2. Root system analysis of *rhp1.e* mutant and WT cv. 'Sebastian' at 14-day seedling stage. **(A)** The longest seminal root in mutant and WT. Root system parameters of *rhp1.e* mutant and WT plants: **(B)** Number of seminal roots, **(C)** Total length of roots, **(D)** Total surface area of roots and **(E)** Total root volume. Significant differences between *rhp1.e* mutant and WT were estimated using Student's t-test. Graphs show mean value of four biological replicates (each replicate represented six plants per genotype) with SD.

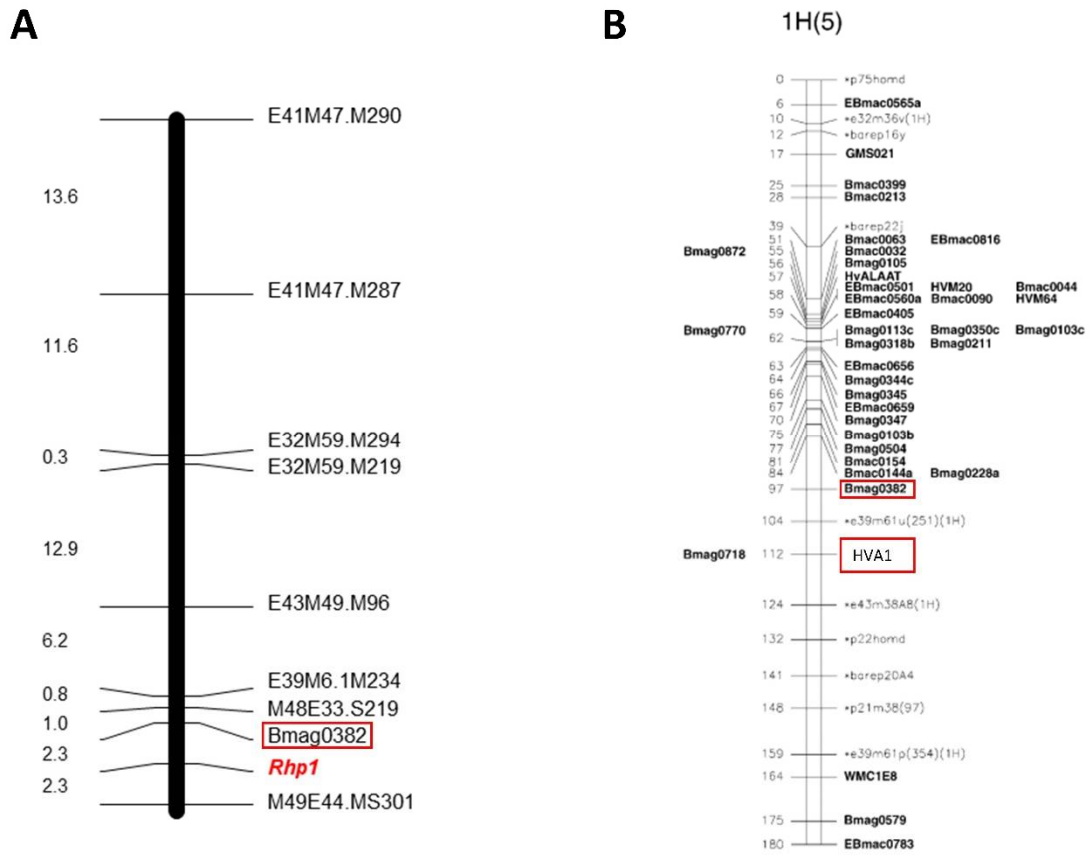


Figure S3. Location of *rhp1* gene on barley chromosome 1H. **(A)** Linkage group of *rhp1* gene on chromosome 1H based on the *rhp1.b* x 'Morex' mapping population (from Chmielewska et al., 2014 [41]), **(B)** Molecular linkage map of barley of 'Lina' x *Hordeum spontaneum* Canada Park population (based on Ramsay et al., 2000 [44]).

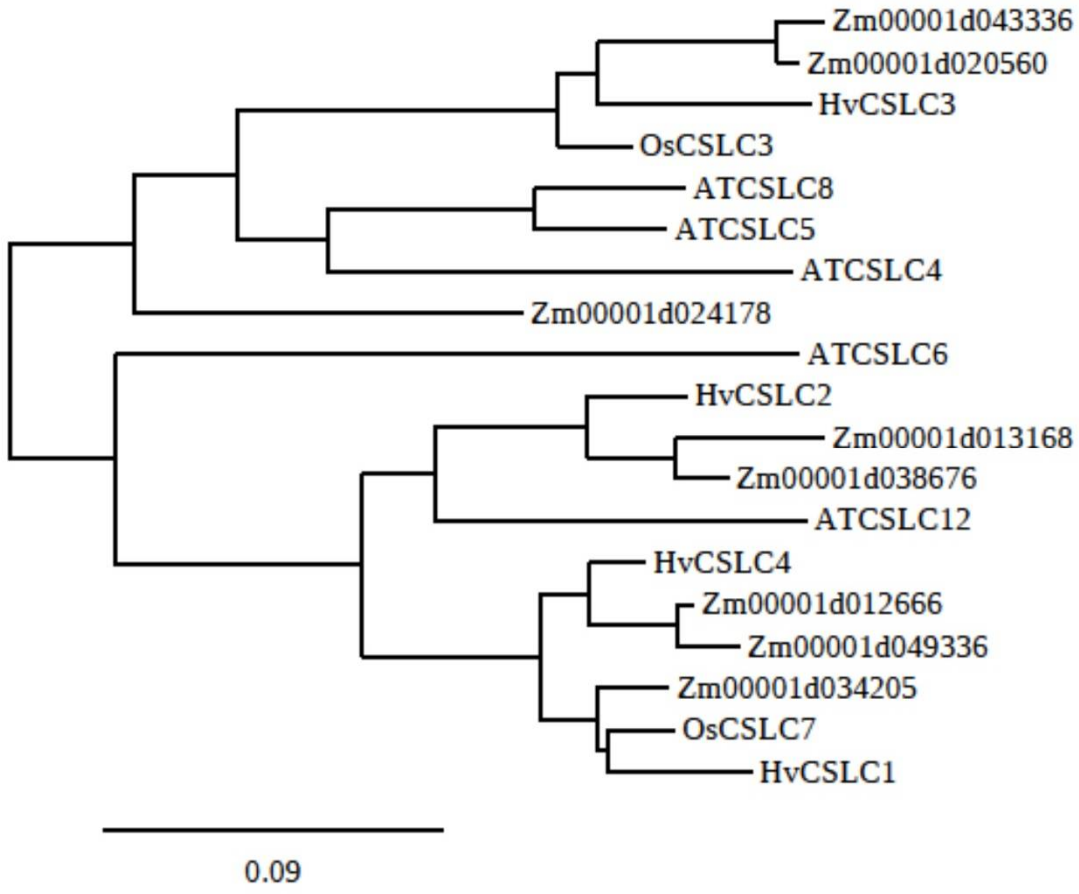
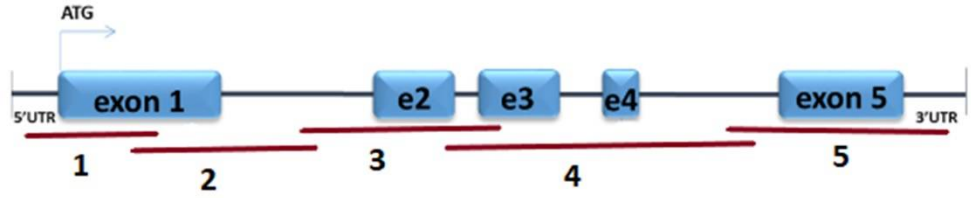


Figure S4. Phylogenetic analysis of CSLC members expressed in the root of *A. thaliana* (AT), *Z. mays* (Zm), *O. sativa* (Os) *and H. vulgare* (Hv). The tree was generated using Phylogeny.fr (www.phylogeny.fr) in "OneClick" mode [93]. This pipeline connects the following programs: MUSCLE for multiple alignment, Gblocks for automatic alignment curation, PhyML with the approximate Likelihood Ratio Test of branch support for tree building and TreeDyn for tree drawing.

A



B

Primer sequence	Ta	Amplified fragment No.
Forward CTCGTTTCGGTTTGACACG Reverse ATCTCGGGCATCTCAAGGT	56	1
Forward GCTTCTACGGCTGCCTCA Reverse ACCGGTGACGACAAACTTCT	56	2
Forward TCCAATCACGACTCTGATGC Reverse CGAAGTGGAAGCACAGGTTT	56	3
Forward CAAGGTTTGACTCGACTGAGC Reverse AGATGTGCTGGCATGAATTG	56	4
Forward CACCCTCTTATACTCAAGCAGTCA Reverse CCCGTCCAAAGGGTTTAGAT	56	5

Figure S5. (**A**) The structure of the candidate gene *HORVU1Hr1G077230* and fragments used for its amplification. (**B**) The list of primer sequences used for PCR amplification of the *HORVU1Hr1G077230* gene in *rhp1* mutants. Ta - annealing temperature

Table S2. The list of primer sequences used in qPCR analysis. Ta - annealing temperature

Gene	Primer sequence	Ta
HvCSLC1	Forward ATCGTCCCGTACCTCCTCTT Reverse CTTCTTGGTGACCACCCACT	56
HvXXT1	Forward GGTCGAGTTCTTCTGGTGGA Reverse TGTCGTCGTAGACCATCTCG	56
HvMUR3	Forward AATCTGACTGGGGCAACAAC Reverse CTTTGGCAGGGTGGAAGTAA	56
HvXLT2	Forward CTGCTCGCCATGTGTGATAC Reverse CGACAAGCTGGTCCCTTC	56
HvMUR2	Forward GGCAACATGCTCAAGAACAA Reverse TCGTGCTCGAGGTAGACGTA	56
HvAXY4	Forward CACCGTCTCCATCTTCTGGT Reverse TCGAGGAACACGTTGTTGTG	56
HvAXY4L	Forward CTCTACTCGTCCCGGAGCTT Reverse GATCGTCGTCATCCTCTGGT	56
HvXTH14	Forward ACACCAACGTGTATGCCAGA Reverse TGGGTCCAAATGATGCTGTA	56
HvCSLC3	Forward ATGAGCTGCGACTACGTCAA Reverse AGGTACGGTGAGCTTGAGGA	56