SUPPLEMENTARY MATERIAL

EXPERIMENTAL ARTICLE

INFLUENCE OF MUTATIONS IN GLYCANASE GENES ON THE FORMATION OF EXO-OLIGOSACCHARIDES IN NODULE BACTERIA *RHIZOBIUM LEGUMINOSARUM* BV. *VICIAE* VF39

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Table 1S. Primers used in this study

Primer	Sequence (5' →3')	Introduced restriction site
plyCf	TTGAATTCATCCACCTGGATGGCTTG	EcoRI
plyCr	TTGAGCTCGATGACGATGTTCGATG	SacI
plyCf2	TTGAGCTCCTCCGACCTGGAC	SacI
plyC_r4	TTTTCTAGAGGCTCAAACGGAGTCG	XbaI
plyB_f	TTGAATTCGAGCGCAAGCGAAG	EcoRI
plyB_r	TTACTGCAGTTTCAAGTTTTCCACCG	PstI
plyB_f	TTGAATTCGAGCGCAAGCGAAG	EcoRI
plyB_r	TTGAGCTCGATCCAGAAGCCGTC	SacI
plyC_f3	TTGAGCTCGGCCAATGGAACCCT	SacI
plyC_r2	TTTAAGCTTCGCATGGCGAAGCC	HindIII

Table 2S. Viscosity and concentration of polysaccharides in the culture medium of the *Rlv* VF39 strain and its derivatives with mutations in glycanase genes

Strain	Culture medium viscosity, cP	Relative culture medium viscosity	High-Molecular-Weight EPS, μg ml^{-1}	Low-Molecular-Weight EPS, μg ml^{-1}	
				Neutral	Acidic
VF39, wt	40 ± 4	1	1059 ± 37	16.5± 2.0	106.5 ± 9.8
plyB	63 ± 17	1.6	1190 ± 38	79.5± 10.0	72.4 ± 21.4
plyBC	170 ± 4	4.3	995 ± 163	5.7± 2.6	62.4 ± 23.8
pssW	109 ± 2	2.7	1181 ± 188	79.8±2.9	66.6 ± 1.85
pssWplyB	128 ± 18	3.2	1153 ± 186	124.8± 19.0	46.9 ± 2.2
pssWplyBC	177 ± 8	4.4	1235 ± 149	179.7± 21.5	44.5 ± 5.2
pssE	–	–	28 ± 2.6	273.0± 6.9	18.0 ± 0.7

Table 3S. Acidic exo-oligosaccharides* of the *Rlv* VF39 strain and its derivatives with mutations in glycanase genes

Strain	Acidic EOS, μg	Distribution of acidic EOS by chromatographic peaks, μg			
		M1	M2	M3	M4
VF39, wt	2437	495	582	70	903
plyB	2094	400	519	66	789
plyBC	1726	–	–	–	527
pssW	1606	411	242	50	558
pssWplyB	748	150	98	–	127
pssWplyBC	1021	134	–	–	188

* the data of ion-exchange fractionation of low-molecular-weight exopolysaccharides (3.5 mg) on a MonoQ 5/50 GL column are presented.

Table 4S. Molecular masses of octasaccharide trimers and *m/z* values of triply charged negative ions of oligosaccharides from the S6 fraction of exo-oligosaccharides of the *Rlv* VF39plyB strain according to NSI mass spectrometry data

THb_0	M_w	*m/z*
Ac_0	4393.17	1463.39
Ac_1	4435.18	1477.39
Ac_2	4477.19	*1491.40
Ac_3	4519.20	*1505.40
Ac_4	4561.21	1519.38
Ac_5	4603.22	1533.38
Ac_6	4645.17	1547.39

THb_1	M_w	*m/z*
Ac_0	4479.13	*1492.04
Ac_1	4521.14	*1506.05
Ac_2	4563.15	1520.05
Ac_3	4605.16	1534.05
Ac_4	4647.17	1548.06
Ac_5	4689.18	1562.06
Ac_6	4731.19	1576.07

THb_2	M_w	*m/z*
Ac_0	4565.17	1520.72
Ac_1	4607.18	1534.72
Ac_2	4649.19	1548.73
Ac_3	4691.2	1562.73
Ac_4	4733.21	1576.74
Ac_5	4775.22	1590.74
Ac_6	4817.23	1604.75

THb_3	M_w	*m/z*
Ac_0	4651.21	1549.40
Ac_1	4693.22	1563.40
Ac_2	4735.23	1577.41
Ac_3	4777.24	1591.41
Ac_4	4819.25	1605.41
Ac_5	4861.26	1619.42
Ac_6	4903.27	1633.43

- not detected. T is a trimer of the octasaccharide unit; M_w is the molecular mass; * trace amounts. Ions with similar masses (highlighted with one color) are located in the same spectral packet.

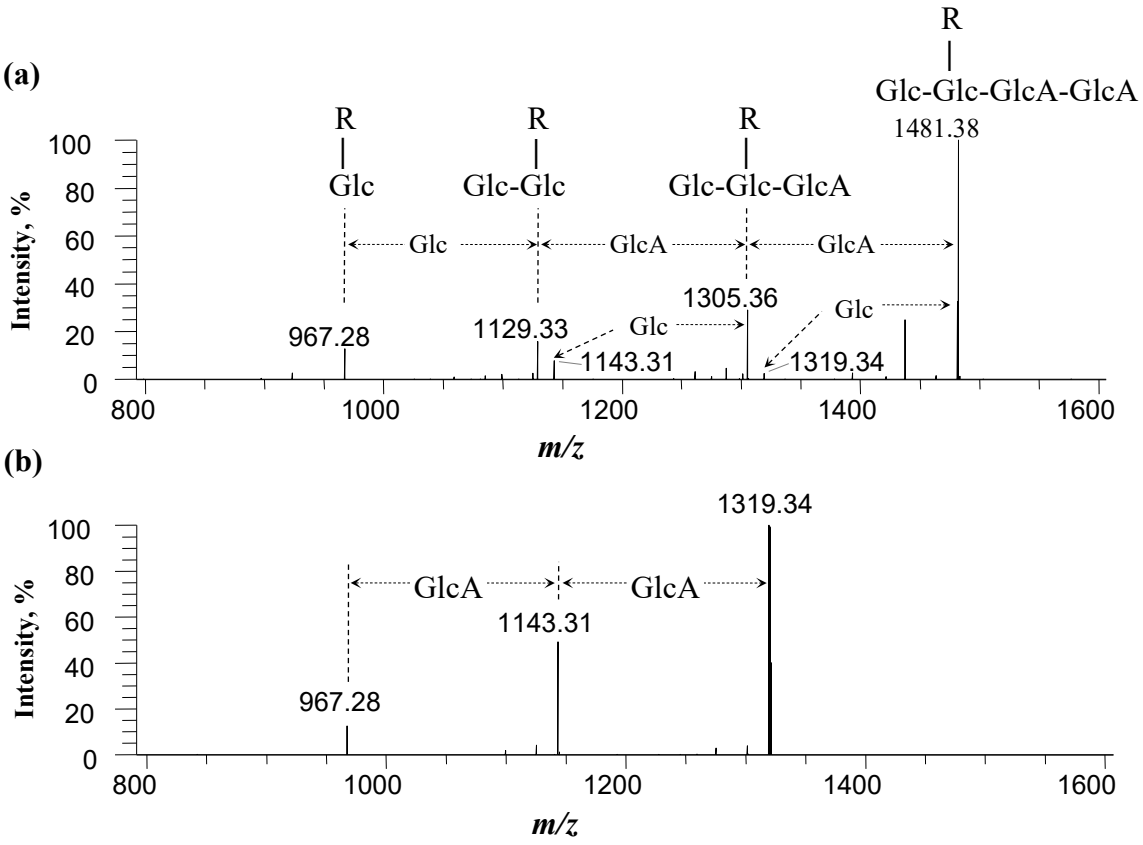


Fig. 1S. CID fragmentation of octasaccharide ion M_8^h (*m/z* 1481.38) **(a)** and heptasaccharide ion $^bM_7^h$ (*m/z* 1319.34) **(b)** from the S4 fraction of oligosachcharides of the *Rlv* VF39 strain with a mutation in the *plyB* gene. R is the side chain of the octasaccharide.

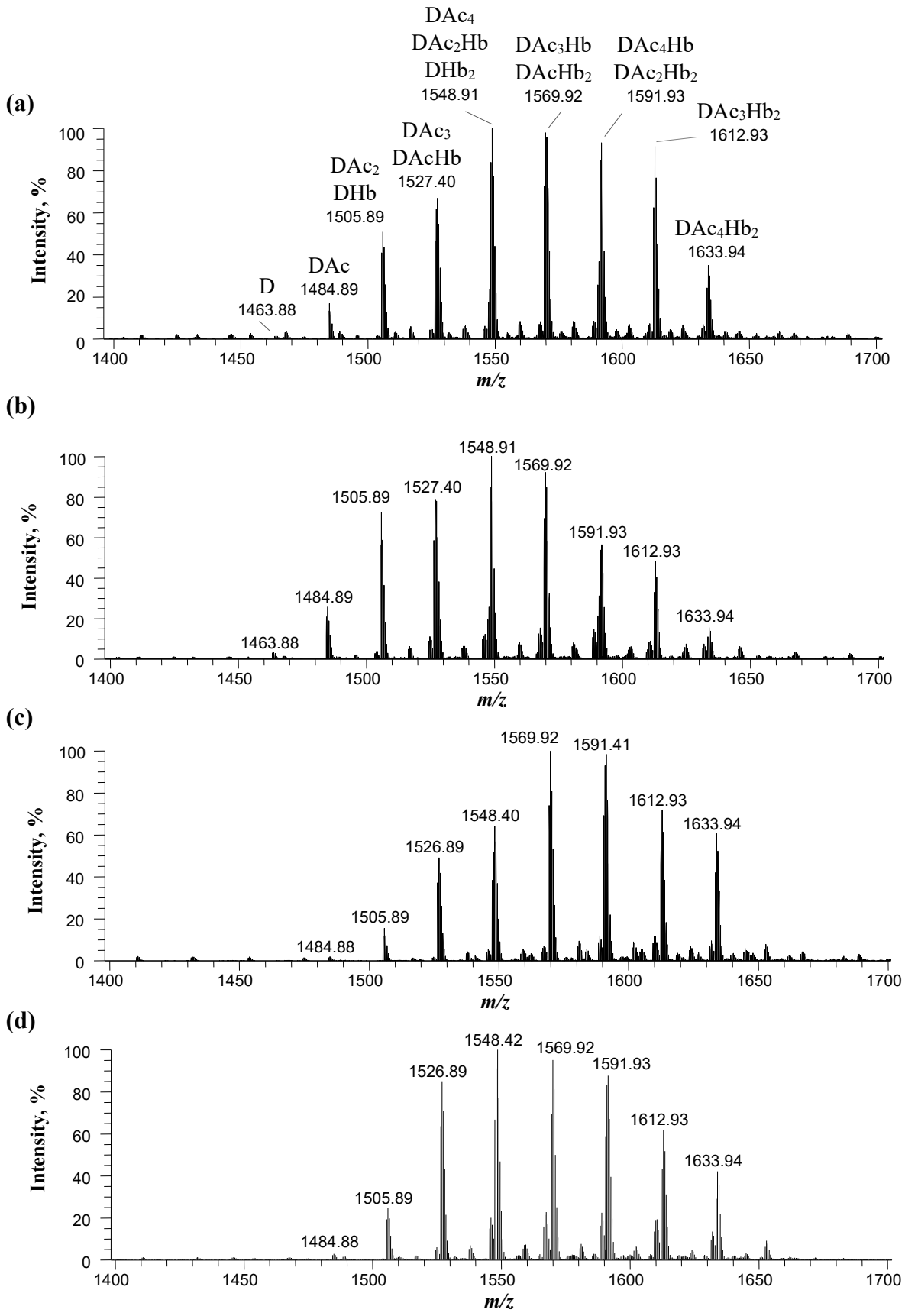


Fig. 2S. Fragments of the NSI mass spectra of exo-oligosaccharides of the *Rlv* VF39 strain with mutations in *pssW* (fraction S2) **(b)**, *plyB* (fraction S5) **(c)**, and *pssWplyB* (fraction S8) **(d)** genes in comparison with the spectrum of dimeric forms of the wild type strain octasaccharide **(a)**. The region of doubly charged negative ions with *m/z* 1400–1700 is shown.

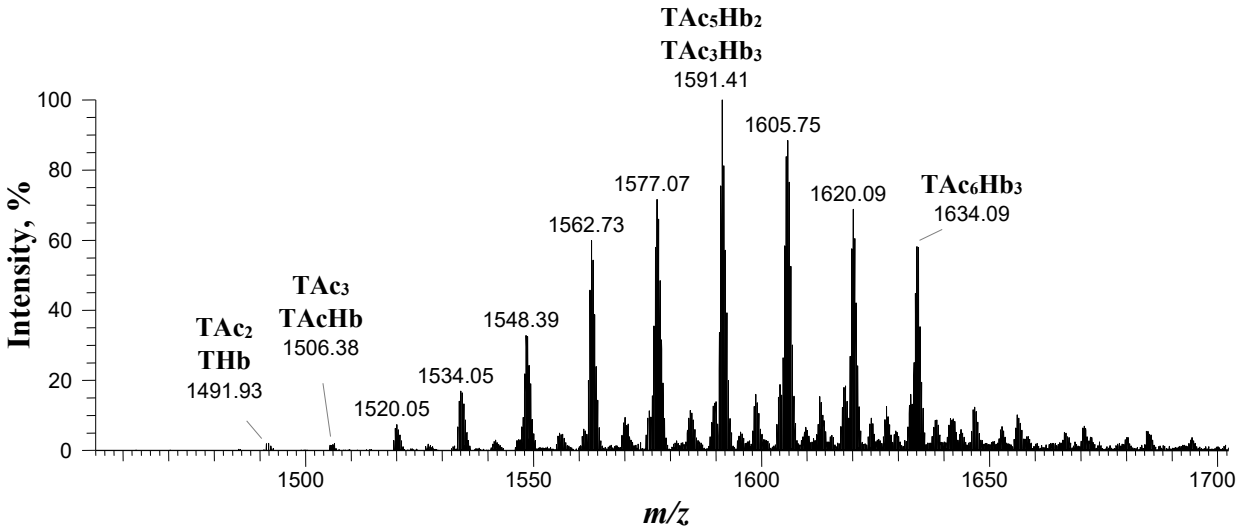


Fig. 3S. A fragment of the NSI mass spectrum of oligosaccharides from the S6 fraction of exo-oligosaccharides of the *Rlv* VF39 strain with a mutation in the *plyB* gene. The region of triply charged negative ions of trimers of the EPS octasaccharide unit with varying contents of Ac and Hb groups is shown. The full spectrum decoding data are presented in Table 4S.

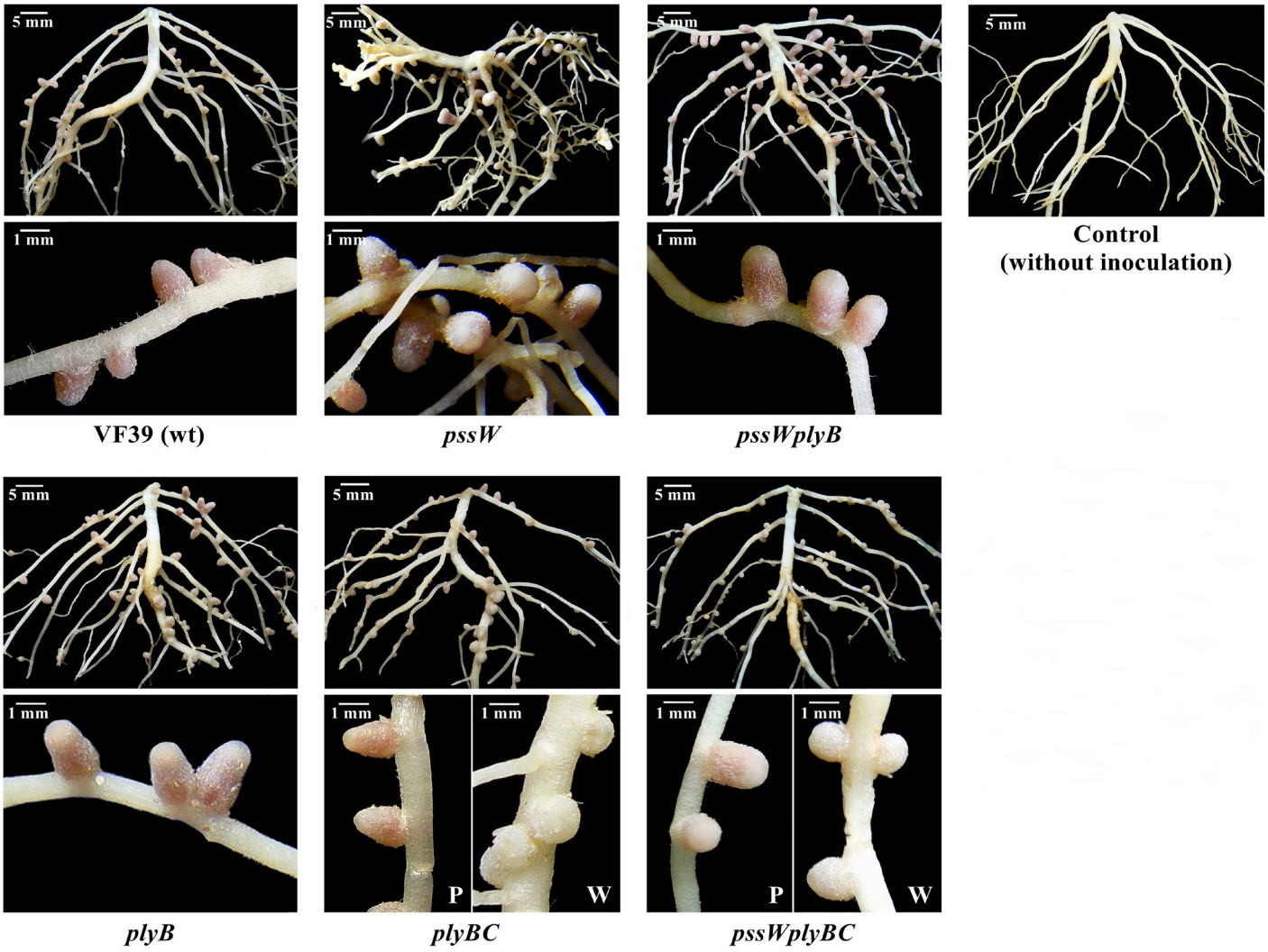
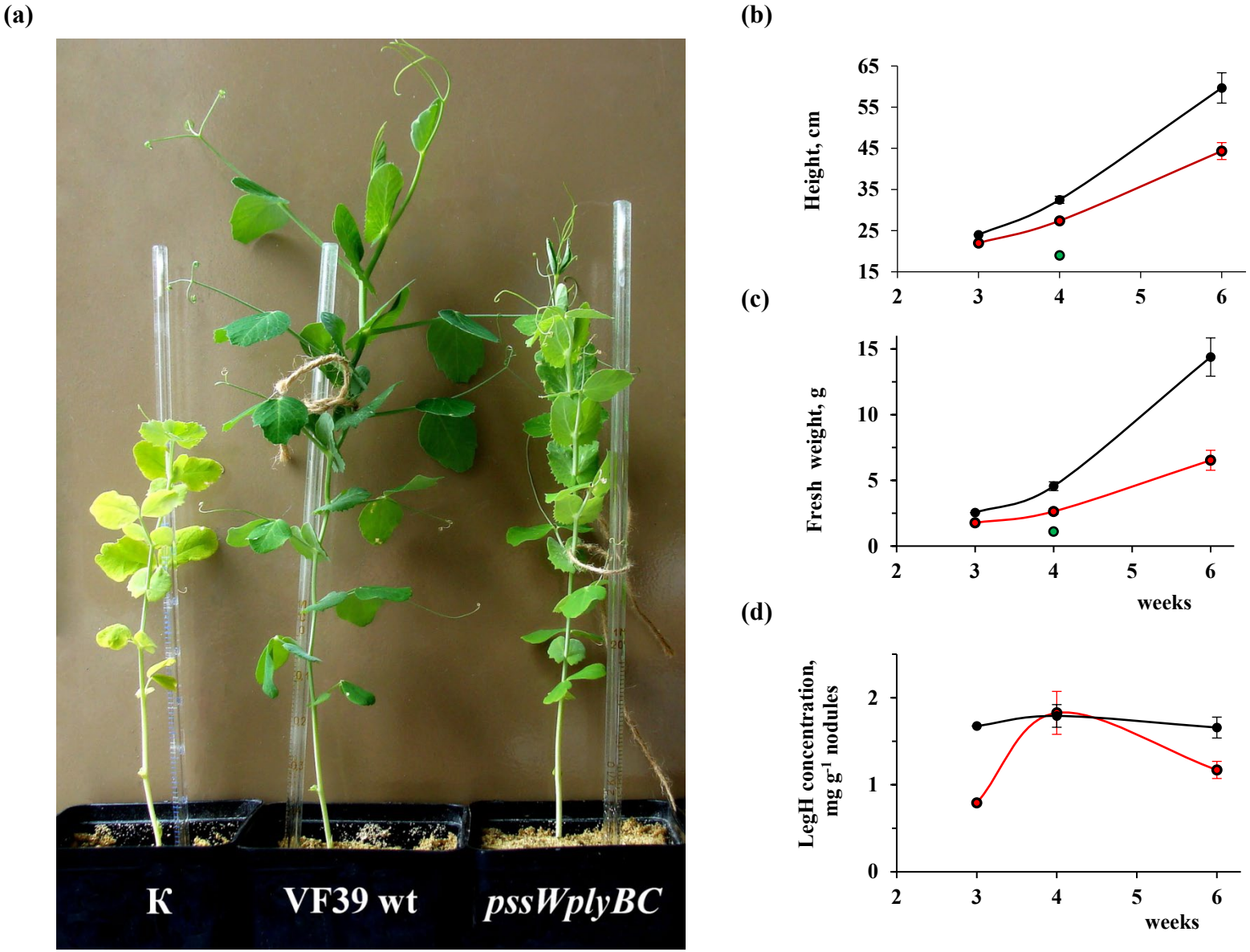


Fig. 4S. Nodules in the stem zone of pea roots 28 days after inoculation with the wild type *Rlv* VF39 strain and its derivatives with mutations in glycanase genes. Pink (**P**) and white (**W**) nodules.



5S. (a) *P. sativum* plants 4 weeks after inoculation with *Rlv* VF39 (wild type) and *pssWplyBC* strains. K – without inoculation. Height **(b)**, fresh weight **(c)** of plants and leghemoglobin (LegH) concentration in nodules **(d)** 3, 4 and 6 weeks after inoculation. (—●—) - *Rlv* VF39, (—●—) – *pssWplyBC*, (●) - control (without inoculation).