

Table S1. *In silico* analysis of primer specificity to yellow dwarf virus (YDV) sequences in NCBI GenBank

Primer name	No. of sequences in the analysis¹	Top off-target match²	No. of base matches in primer³	Matches in 3' end of primer⁴	No. of base matches in partner⁵	Matches in 3' end of partner	Length (bp) between primer pair⁶
SGVF2	92	PAS	25/26	3/4	8/20	0/4	N/A
SGVR	92	GPV	9/20	0/4	8/26	0/4	N/A
RPVL	87	RPS	21/21	4/4	9/24	0/4	N/A
RPVR	87	GPV	19/24	0/4	20/21	4/4	186
PAVL	34	MAV	15/20	0/4	15/20	4/4	Null
PAVR	34	PAS	17/20	4/4	8/20	0/4	N/A
MAVF	91	GAV	20/24	1/4	23/27	1/4	362
MAVR	91	GAV	23/27	1/4	20/24	1/4	362
RMV-MTF	92	RPS	18/24	3/4	10/26	0/4	147
RMV-MTR	92	RMV	20/26	1/4	19/24	2/4	147
PASF1	87	MAV	26/27	3/4	11/23	0/4	440
PASR1	87	PAV	18/23	3/4	26/27	3/4	444
TaUbiqF	92	RPV	11/19	0/4	8/20	1/4	N/A
TaUbiqR	92	PAV	9/20	0/4	9/19	0/4	N/A

¹GenBank sequences excluding those of the intended YDV species target, and including the wheat ubiquitin internal reference sequence (TaUbiq). ²most significant match using NCBI nucleotide BLAST (Blastn) for short oligos. ³Number of bases along the entire length of the primer that matched the top off-target YDV sequence. ⁴last four bases at the 3' terminal end of the primer; must match 100% for the Taq polymerase for efficient polymerization to extend beyond the 3' end of the primer (Dieffenbach et al., 1993; Wu et al., 1989). ⁵Number of bases along the length of the primer partner (forward or reverse of the primer indicated) that matched the top off-target YDV sequence indicated. ⁶The length of the top off-target template demarcated by the forward and reverse primers in the primer pair; N/A = both primers in the pair are in the incorrect orientation on the template to produce a product.