

Hi Alison.

I screened them with my own Kaspars and there do seem to be a few problems I'm afraid.

Here is my initial take on my findings:

I validated my primers on the parental accessions, background accessions, and some PpdB1 and PpdD1 NILs from Dave Laurie and myself and they worked fine.

In a second experiment I ran them across 5-6 plants each of the seed you gave us (which we are glasshouse bulking) and in summary:

PpdA1a SHW NILs – all came back as **WT (PpdA1b)** (see note 1 below). This applies to:

GRU #	NIAB CODE
10245	A-32/ASHW-131-2
10247	AL-173-B1-B-8-8
10262	ROB-173-2-A-17-7
10264	ROB-173-2-A-17-1
10265	ROB-173-16-A-10-7

PpdB1a CS NILs –

GRU #	NIAB CODE	Kaspar Score
10237	A/Ach-151-1-6	Segregation: WT (PpdB1b) and PpdB1a
10239	ALCS-A-ACH-151-2-11	WT (PpdB1b)
10257	ROBCS-R/RC-211-4	PpdB1a

PpdB1a Timstein NILs cannot be tested by Kaspar as no SNP exists between D1a and D1b alleles in Alchemy and Robigus (need CNV assay).

10237 is currently undergoing purification, plant 5 passes the Kaspar marker test and will be bulked.

PpdD1a C67 NILs –

GRU #	NIAB CODE	Kaspar Score
10241	A/AC-121-8	WT (PpdD1b)
10243	A/AC-131-3	PpdD1a
10259	R/RC-211-2-4	WT (PpdD1b)

PpdD1a Soissons NILs –

GRU #	NIAB CODE	Kaspar Score
10249	A-11/AS-212-1-6	PpdD1a
10251	A-12/AS-212-1-1	PpdD1a
10266	R-34/RS-231-4-4	PpdD1a
10268	R-35/RS-111-2-3	WT (PpdD1b)

Note 1 – PpdA1a assay – this was designed to detect the 1117bp deletion (GS105) and has only been validated against GS105 itself (tetraploid) and not against any hexaploid NILs. I will double check these lines using the “Old Style” PCR markers later in the summer. That said, there seems to be something odd about the phenotypes with these lines. The correct PpdD1 pairs show a phenotype shift of 2-3 days which is about right for natural daylength, whereas the Robigus PpdA1 pairs show a shift of 6-7 days and the Alchemy ones 12 and 19 days respectively – much too big for Ppd under natural daylength conditions, in my opinion.

Note 2 – All late controls came back as having the correct (Ppd1b) allele (excluding PpdB1b Timstein for which no Kaspar is available)

My feeling is that the PpdB1a and PpdD1a results are genuine. There is an outside chance that 10237 and 10239 results are due to only detecting the allele in Robigus and not Alchemy but this seems unlikely to me.

Sorry to be the bearer of bad news. The plants still have green tissue so if you want me to sample them again for you and send you the tissue I can do that.

Best Wishes
Adrian