

DFW 2017 Breeders' Toolkit (BTK2017)

JIC WISP landrace pillar NILs Cohort 1 (BC3 equivalent)

Following on from WISP, the 'Designing Future Wheat' (DFW) programme continues to evaluate some Near Isogenic Lines of wheat landrace QTL regions in the genetic background of Paragon. The landrace NILs were developed through two marker assisted backcrosses that started off with a RIL from the respective Paragon x Watkins landrace population. So the NILs carrying Watkins alleles will carry ~ 87% Paragon background.

The NILs are derived from the first cohort of landrace derived NILs from WISP. The emphasis is thus on height and heading as the QTL analysis to inform the choices were based on data from the beginning of WISP at JIC. The outcome of the BTK2016 trials confirms that the three tested landrace alleles carry agronomic benefits in an 'Additive Main effects and Multiplicative Interaction' (AMMI) analysis. The AMMI model is one of the most widely used statistical tools in the analysis of multiple-environment trials. It has two purposes, namely understanding complex GEI and increasing accuracy. These are the results for nine sites (3 research institutes, 6 breeders and a 10th site excluded due to lodging, with 'Paragon' mean GRYLD = 8.61 t/ha):

NIL stream	QTL	GRYLD [t/ha] landrace allele in QTL regions	GRYLD [t/ha] 'Paragon' allele in QTL region	Advantage (% increase)
PW141-7	7B-Ht	8.51	8.43	0.89
PW352-54	3A-EM	8.4	8.27	1.61
PW729-77	5A-EM	8.58	8.04	6.71

(A more detailed outcome for the AMMI analysis of the BTK2016 yield trial is at the end.)

Grown in the same trials, but only at the three research institutes (JIC, RRes, SB), we have selected two new landrace NILs for the 2017 Toolkit:

NIL stream	QTL	GRYLD [t/ha] landrace allele in QTL regions	GRYLD [t/ha] 'Paragon' allele in QTL region	Advantage (% increase)
PW141-16	2D-EM	8.96	8.79	1.92
PW034-19	2B-EM	8.69	8.50	2.17

New for 2017-18 BTK trials is input from DFW WP1 and is added to the above two streams:

WP1EMS mutant derived NIL for GW2-A1 (BC4 equivalent)

TaGW2-A1 acts as a negative regulator of grain weight and size. An EMS (Ethyl Methane Sulfonate)-treated population of the tetraploid wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) var. Kronos [Uauy et al 2009]) was screened for mutations in the *TaGW2-A1* gene. TILLING line T4-2235 contains the G2373A splice site mutation which results in a frame-shift that generates a premature termination codon 27-bp within exon 5. T4-2235 was backcrossed four times to wild-type non-mutagenized Paragon to generate BC4F2 lines segregating for the wild type/mutant alleles (97% identical).

Description of the BTK2017-18 material

In all seven accessions, three of them parental donors, are included the 2017-18 BTK. The set contains three accessions with either a 'new' Watkins allele or a mutant allele, respectively, (see table below) and their Paragon counterparts. A control Paragon variety makes up the set. Data from the lines carrying the Paragon allele is useful as it provides the best comparison for the Watkins allele effect. In addition, all of these lines carry about 12.5 % random Watkins background. If you identify traits that might be interesting for breeding purposes, the genotyping group (Bristol) would be happy to genotype these lines to specifically show what background is present.

Origin	NIL stream	QTL / Gene	Landrace/Mutant sib	Paragon/Wildtype sib	Markers used for selection
WP3	PW141-16	2D-EM	12W	10P	BS00003804 BS00069899 BS00021912
WP3	PW034-19	2B-EM	12W	11P	BS00064155 BS00074661
WP1	TILLING line T4-2235	<i>GW2-A1</i> a	mutant (A)	wildtype (G)	TaGW2_A_F_specific TaGW2_A_R_wildtype TaGW2_A_R_mutant

Please plant three reps for each allele, or more if possible. Each locus comes with two sibs, which will make 18 plots. Please include the Paragon controls (at least three reps, which will make it 21 plots). The material is best sown in the middle two weeks of October and treated with your fully treated agronomy approach. Seed is sent untreated as we appreciate you have your own practices here and with a TGW attached to ease workload, although again we appreciate you may have your own approaches to drill rates

Details from the analysis of the BTK2016 trials

Grain yield (GRYLD) data from 10 sites was adjusted for plot size to t/ha and to 15% moisture content. Average GRYLD for each NIL sib was calculated as BLUE using a linear model from the three reps. Overall GRYLD average per site was plotted to compare performance of sites. Due to lodging problems, the RAGT results are unusually low and were treated as outliers.

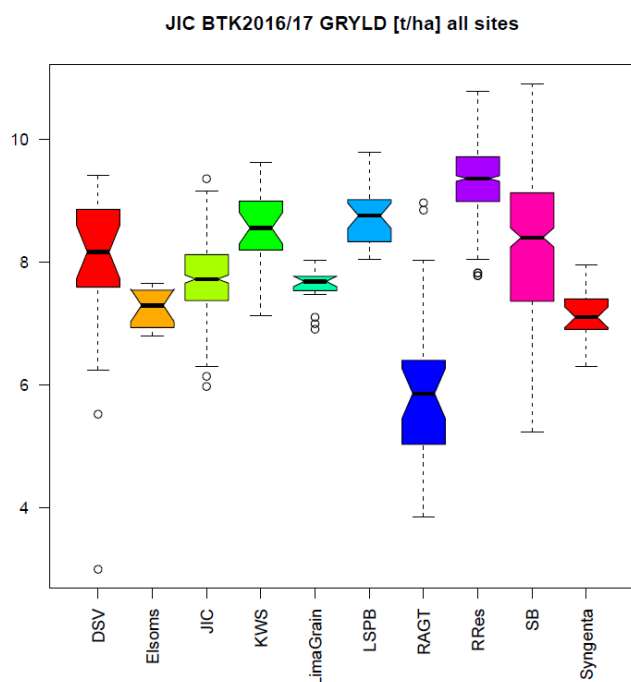


Figure 1: Average GRYLD for each site.

An ‘Additive Main effects and Multiplicative Interaction’ (AMMI) analysis was used to analyse the GRYLD data for all NIL sibs between sites. The AMMI model is one of the most widely used statistical tools in the analysis of multiple-environment trials. It has two purposes, namely understanding complex GEI and increasing accuracy. The AMMI interaction means table gives us an estimate of the average yields for each site and each sib:

sites	Plot size [m ²]	GRYLD [t/ha] (15%moisture) interaction means from AMMI analysis											paragon	comment
		141-16_2DEM_p	141-16_2DEM_w	141-7_7BHT_p	141-7_7BHT_w	34-19_2BEM_p	34-19_2BEM_w	352-54_3AEM_p	352-54_3AEM_w	729-77_5AEM_p	729-77_5AEM_w	729-77_5AEM_wh		
DSV	6-7			7.52	9.06			7.58	8.38	7.45	6.89		8.04	Take All
Elsoms	15			7.15	7.47			7.35	6.83	6.88	7.17		7.43	
JIC	6	7.52	7.88	7.58	8.18	7.91	8.09	8.03	8.03	7.75	8.05	7.74	7.92	
KWS	6			7.75	8.02			8.74	8.96	9.18	8.43		8.57	no moist. data

LimaGrain	6			7.53	7.8				7.79	7.18	7.71		7.61	spring drilling
LSPB	6			9.07	8.69			8.22	8.55	8.54	8.43		9.02	
RAGT	6			5.16	5.46			5.18	5.85	7.62	6.22		6.13	lodging
RRes	6	9.53	9.69	9.38	10.01	9.32	9.47	8.99	9.33	9.05	9.33	9.34	9.55	
SB	6	8.58	8.58	8.06	8.46	7.47	7.73	7.79	7.75	7.7	8.43	8.06	7.95	
Syngenta	9.75			7.33	7.02			6.89	7.14	7.28	7.31		7.14	France

The AMMI1 plot (Figure 2) shows the grain yield (GRYLD) on the x-axis and the first principle component as an indication of the main variation on the y-axis. The closer sites (in red) and the NILs (coloured for each stream in yellow, beige, green, light blue, blue and purple) are to the PC1 = 0 line, the more stable is the performance of site or line. High yield potential sites and lines are right of the average yield (dotted line near GRYLD = 8.6 t/ha), and low yield potential to the left. Each NIL stream is present as a pair, denoted by an attached ‘_w’ for the Watkins landrace allele or by an attached ‘_p’ for the Paragon allele.

AMMI1 (all sites): GRYLD [t/ha] for NIL-stream-sibs

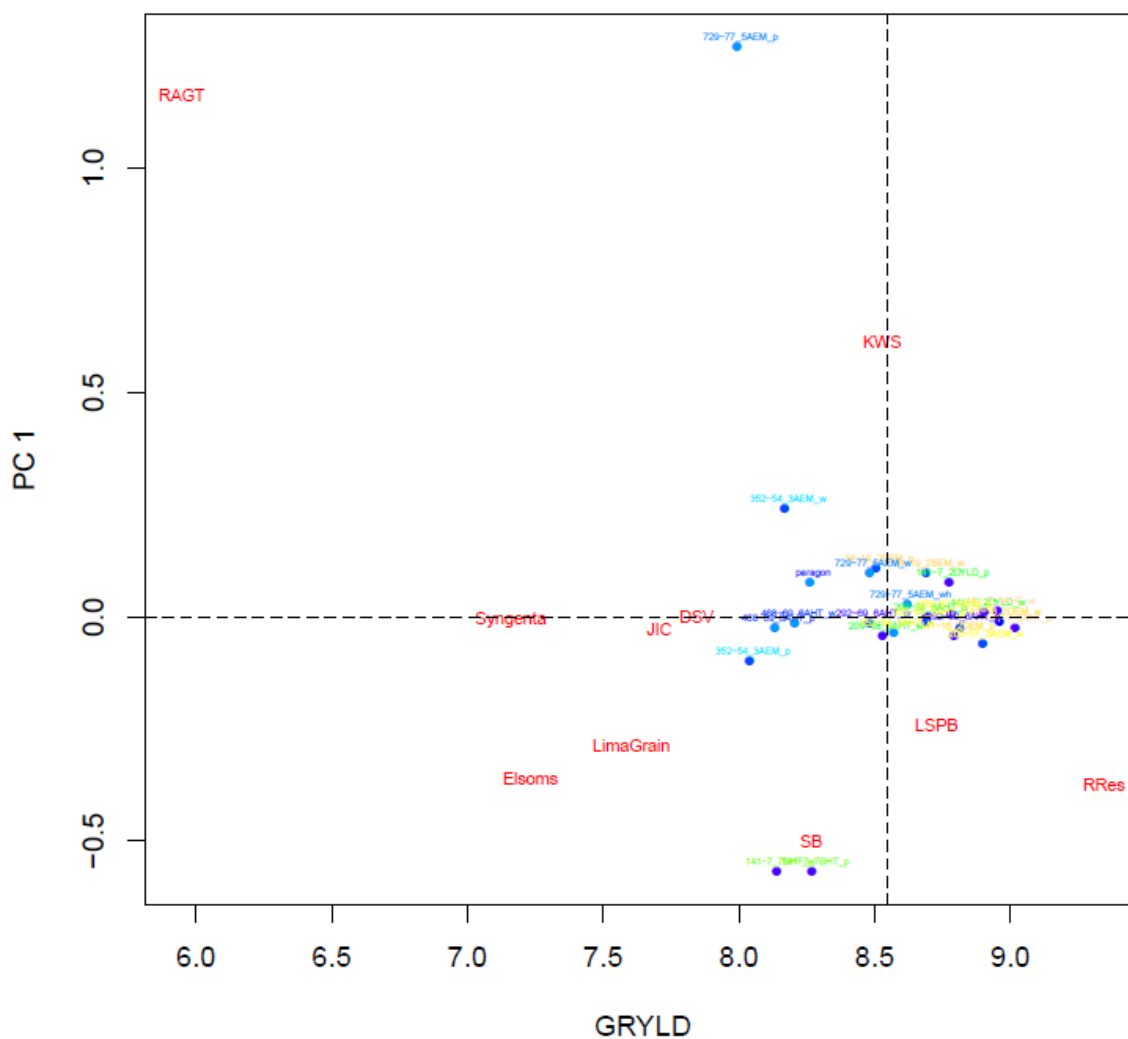


Figure 2: AMMI-1plot for all sites.

The AMMI1 plot in Figure 2 shows the outcome of all sites. However, as the RAGT results, due to

lodging problems, distort the plot. An AMMI1 plot without that site is more suited to judge on the yield potential (see Figure 3). The two blue arrows in the plot show the yield gain between ‘Paragon’ allele and ‘Watkins landrace’ allele of the two selected NIL sibs for the BTK2017 yield trial.

AMMI1 (no RAGT): GRYLD [t/ha] for NIL-stream-sibs

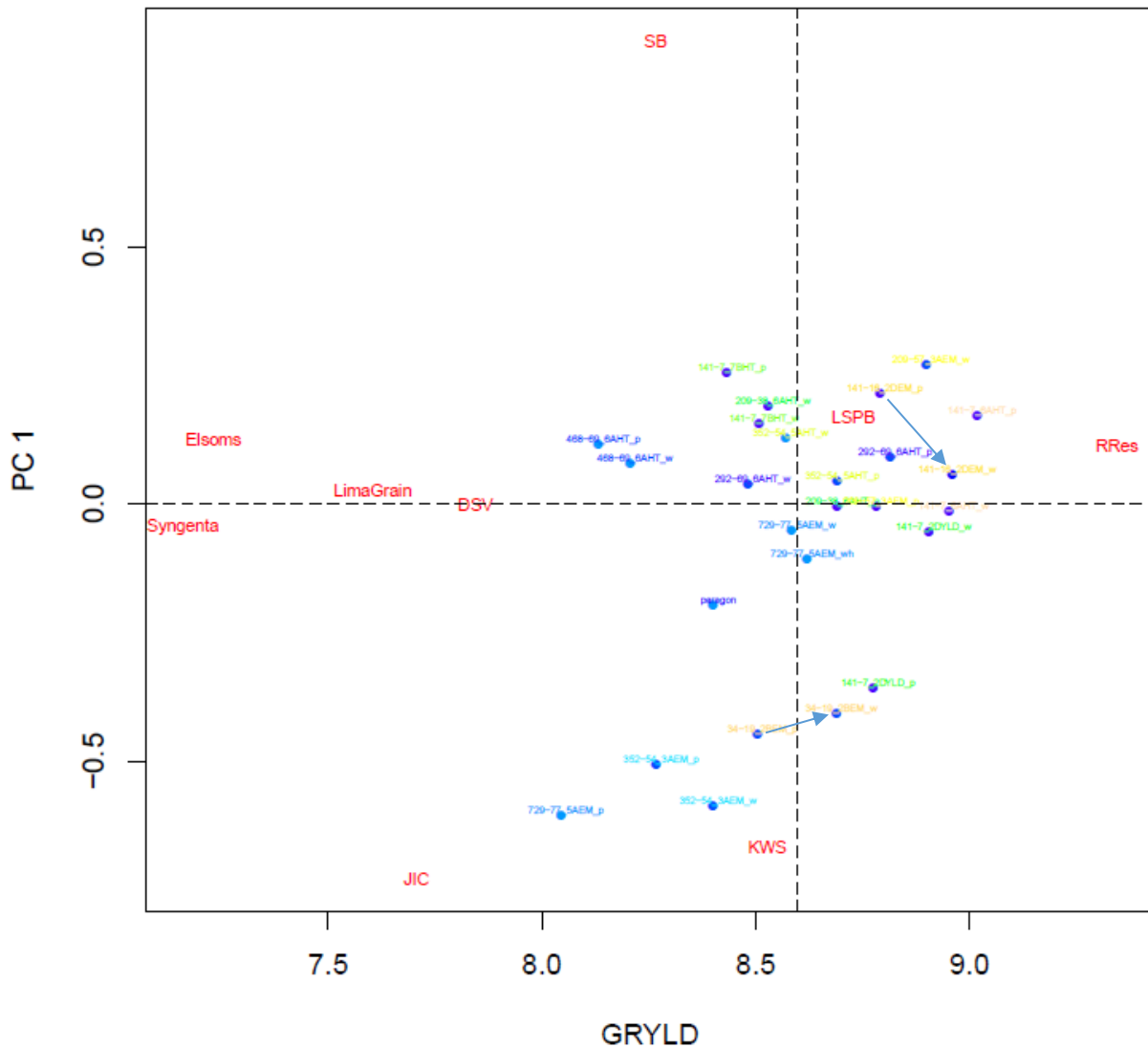


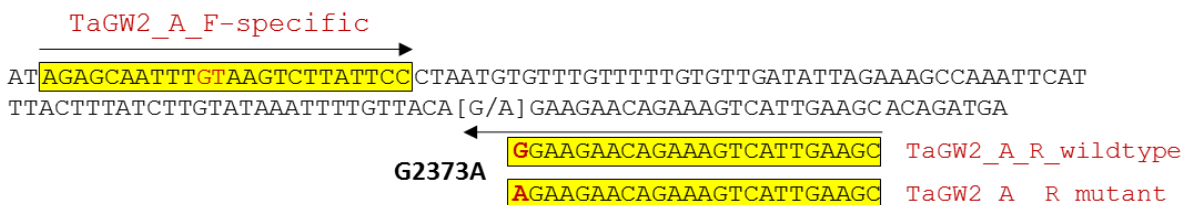
Figure 3: AMMI-1 plot excluding the RAGT results

Details for the TaGW2-A1 NILs

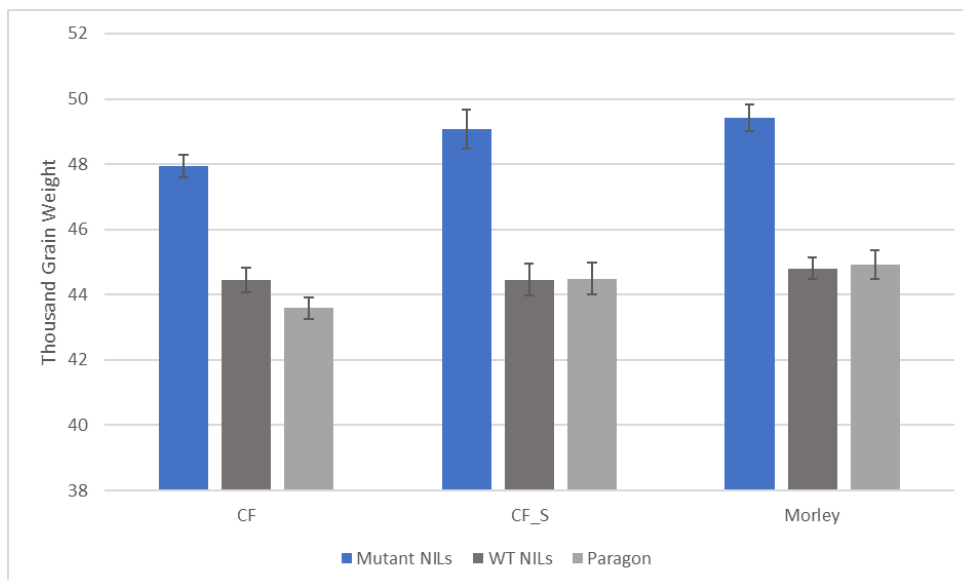
TaGW2-A1 acts as a negative regulator of grain weight and size. An EMS (Ethyl Methane Sulfonate)-treated population of the tetraploid wheat (*Triticum turgidum* L. subsp. *durum* (Desf.) var. Kronos [Uauy et al 2009]) was screened for mutations in the *TaGW2-A1* gene. TILLING line T4-2235 contains the G2373A splice site mutation which results in a frame-shift that generates a premature termination codon 27-bp within exon 5. T4-2235 was backcrossed four times to wild-type non-mutagenized Paragon to generate BC4F2 lines segregating for the wild type/mutant alleles (97% identical).

A KASP assay has been designed to differentiate the two variants of the G2373A SNP. Two allele specific reverse primers were designed with either a G or A at the 3' end: primer K2 for the wildtype (G) and K3 for the mutant (A) allele.

TaGW2_A_F_specific	5'- AGA GCA ATT TGT AAG TCT TAT TCC -3'
TaGW2_A_R_-wildtype	5'- GCT TCA ATG ACT TTC TGT TCT TCC -3'
TaGW2_A_R_mutant	5'- GCT TCA ATG ACT TTC TGT TCT TCT -3'



Three independent trials in 2017 (CF – winter sown low yield potential site; CF_S spring sown low yield potential site; Morley – winter sown high yield potential site) demonstrate the effectiveness of the mutant allele in significantly increasing the size of the grains over the wildtype, with increases in TGW of 7.8%; 10.4% and 10.3% respectively.



TGW(g)	CF	CF_S	Morley
Gw2_A NILs	47.9	49.1	49.4
WT NILs	44.4	44.5	44.8
Paragon	43.6	44.5	44.9

% Increase over WT 7.8% 10.4% 10.3%

Yield was neutral in these trials. Data from CIMMYT on the same allele in US cultivar Kronos suggests yield advantage is observed under stressed conditions during grain filling.

