https://doi.org/10.1071/FP21011_AC

Supplementary Material: *Functional Plant Biology*

Supplementary Material

Concurrent overexpression of amino acid permease *AAP1*(*3a*) and *SUT1* sucrose transporter in pea resulted in increased seed number and changed cytokinin and protein levels

Jan E. Grant[A,H], *Annu Ninan*[B,G], *Natalia Cripps-Guazzone*[A,F], *Martin Shaw*[A], *Jiancheng Song*[B,C], *Ivan Petřík*[E], *Ondřej Novák*[E], *Mechthild Tegeder*[D] *and Paula E. Jameson*[B,H]

[A]The New Zealand Institute for Plant and Food Research Limited, Private Bag 4704, Christchurch 8140, New Zealand.

[B]School of Biological Sciences, University of Canterbury, Christchurch 8140, New Zealand.

[C]School of Life Sciences, Yantai University, Yantai 264005, China.

[D]School of Biological Sciences, Washington State University, Pullman, WA, USA.

[E]Laboratory of Growth Regulators, Faculty of Science, Palacký University, and Institute of Experimental Botany of the Czech Academy of Sciences, Šlechtitelů 27, CZ-78371 Olomouc, Czech Republic.

[F]Faculty of Agriculture and Life Sciences, Lincoln University, New Zealand.

[G]The New Zealand Institute for Plant and Food Research Limited, Auckland, New Zealand.

[H]Corresponding authors. Emails: jan.grant@plantandfood.co.nz; paula.jameson@canterbury.ac.nz

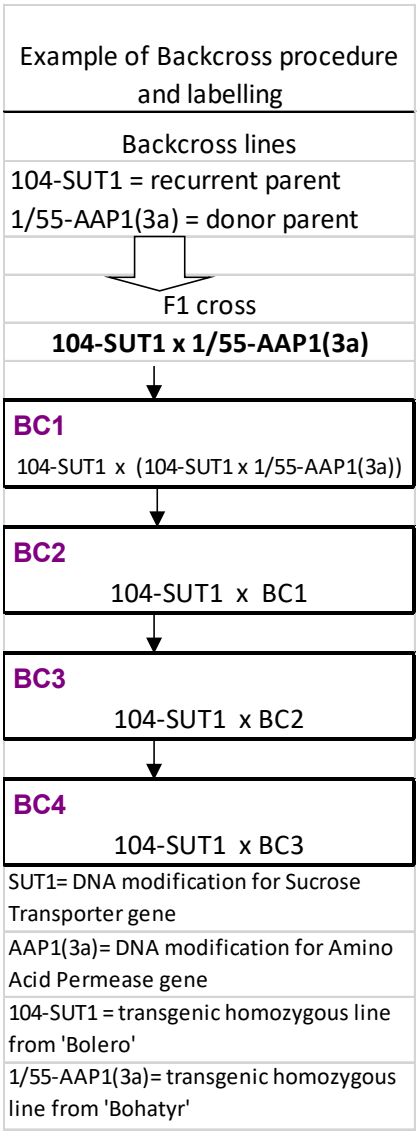


Fig. S1. Schematic for backcrossing procedure. After four backcrosses the plant is calculated to be 96.875% identical to the recurrent parent. To obtain homozygosity of the AAP1(3a) gene a further two rounds of selfing to identify lines containing both genes of the BC4 lines was carried out. At each stage PCR confirmed presence of both genes. After the second round of selfing lines, a minimum of 20 seeds were tested by PCR. All 20 had to be positive for both introduced genes for 99% confidence they were homozygous.

Fig. S2 A: Sucrose Content in cotyledons during development

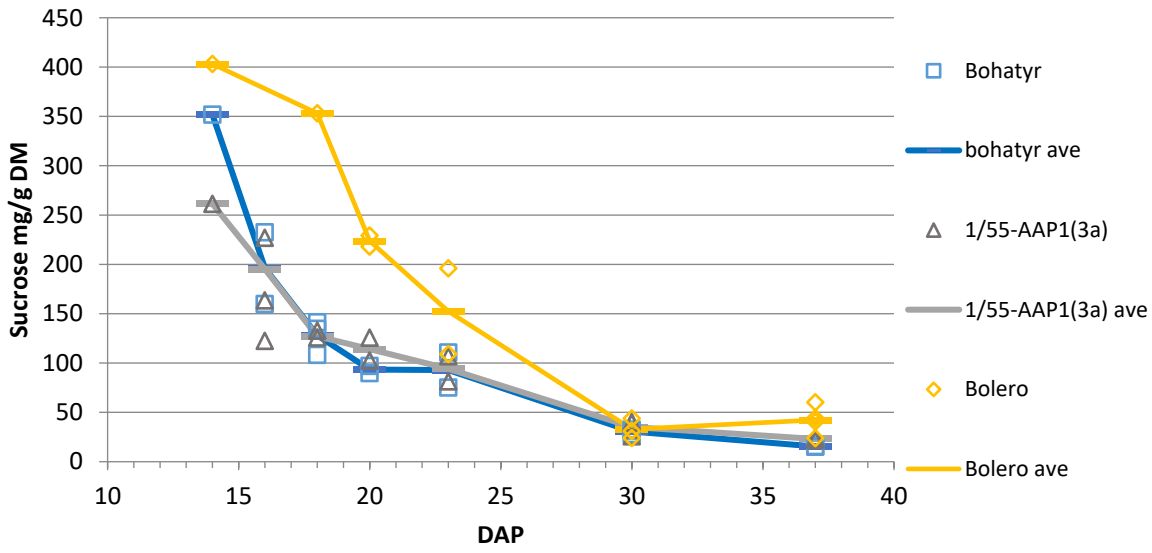
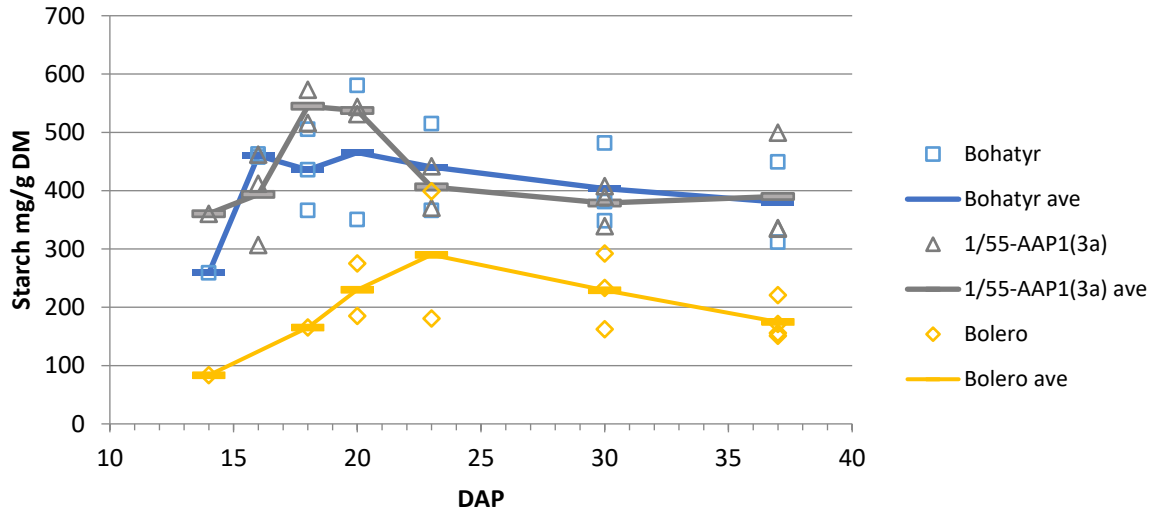


Fig. S2 B: Starch Content in cotyledons during development



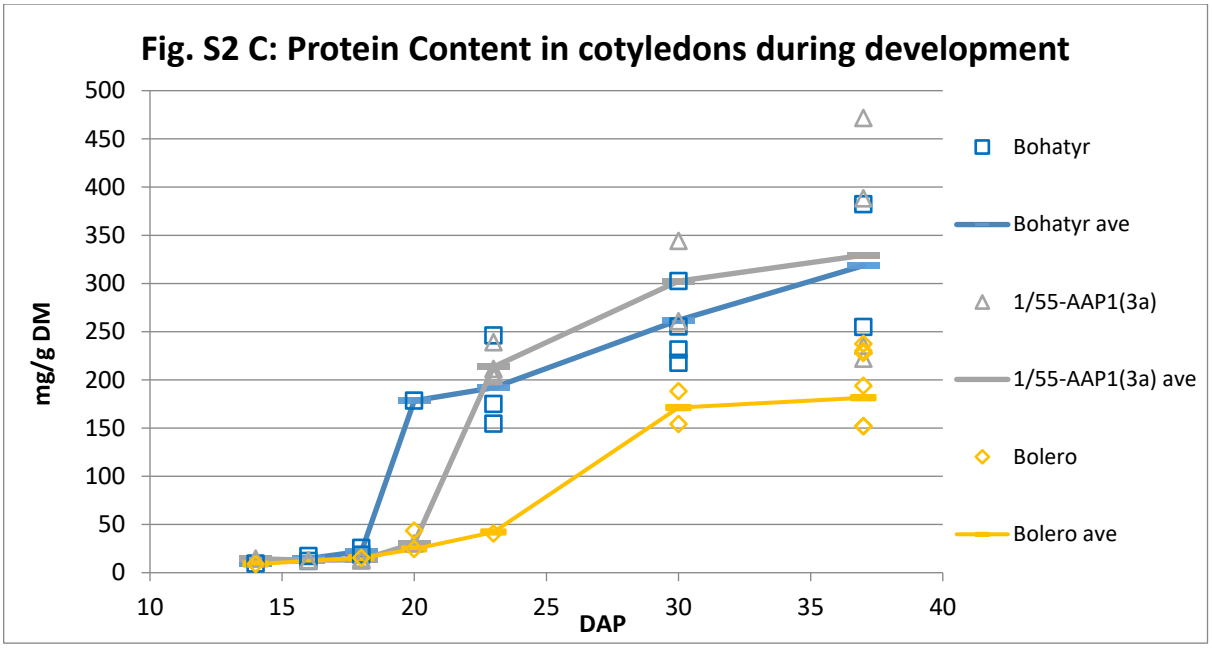


Fig. S2. Biochemical analyses for the donor (1/55-AAP1(3a)) and recurrent (104-SUT1) parents as well as the wildtype 'Bohatyr' field pea line from which the donor line was created.

- **A)** Sucrose content in pea cotyledons during development. The differences are as expected for field ('Bohatyr' and 1/55-AAP1(3a)) and process (104-SUT1) peas.
- **B)** Starch content in cotyledons during development. The high variability meant that there were no significant differences.
- **C)** Protein content during development showing field pea and process pea differences.

Abbreviations: DM = Dry Matter, DAP = Days After Pollination

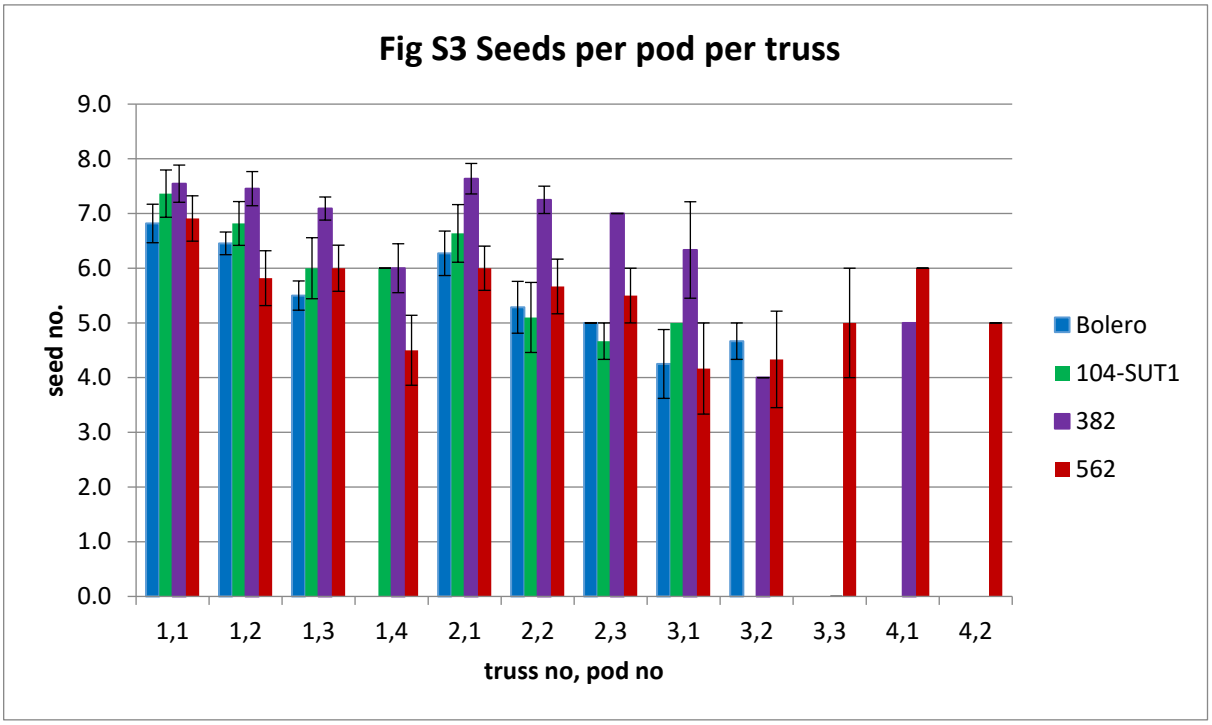


Fig.S3. The number of seeds per pod at each truss on the main branch for wildtype, 'Bolero'; single transgenic line, 104-SUT1; double transgenic lines 382 and 562. This is an average from 11 plants per line with standard error bars. On the x-axis the first number indicates the truss and the second number is pod number on that truss. Pod numbering starts from the base of the truss and moving along the truss. This shows that line 562 set more pods than the other lines while line 382 generally filled an extra seed per pod.

Fig S4A: Line 1/55-AAP1(3a) vs 'BOHATYR' (Leaves)

Target Genes	Developmental stages	
	5d	20d
PsIPT 1 (604)	-2.2	-1.1
PsIPT 2 (605)	-7.4	2.0
PsIPT 4 (421)	-9.1	-1.2
PsCKX 1 (930)	1.4	-11.4
PsCKX 2 (627)	-280.6	-1.1
PsCKX 5 (942)	-2.3	-1.1
PsCKX 7 (910)	4.6	3.3
PsSUT 1 (366 Transgene)	30.9	-1.3
PsSUT 2 (948)	27.0	1.0
PsSUT 3 (674)	4.7	1.1
PsSUT 5 (666)	-15.7	-1.2
PsAAP 1(3a) (532 Transgene)	87.0	2.3
PsAAP 7a (498)(Cluster 1)	2.2	1.6
PsAAP 7b (9261)	1.7	1.4
PsAAP 2a (675)(Cluster 3A)	25.6	2.6
PsAAP 2c (4401)	7.6	1.8
PsAAP 2d (840)	7.0	1.5
PsAAP 3b (051)	8.2	-1.5
PsAAP 6a (931) (Cluster 4B)	-70.2	-1.1
PsAAP 6b (328)	4.6	-1.0
PsAAP 1 (180)	-45.7	-1.1
PsCWINV 1 (240)	-79.2	-1.7
PsCWINV 2 (448)	2.5	1.2
PsCWINV 3 (415)	-14.0	-1.6
PsCWINV 6 (320)	31.3	1.3
PsSW 1 (1) (Clade I)	-1.1	-6.2
PsSW 2a (2a)	-1.2	2.2
PsSW 2b (2b)	8.6	1.4
PsSW 4 (7) (Clade II)	6.1	-1.1
PsSW 5a (6a)	-4.7	-1.1
PsSW 5b (6b)	5.9	-1.7
PsSW 7 (4)	-1.7	1.1
PsSW 12 (13b) (Clade III)	33.5	2.0
PsSW 13 (13a)	4.7	-3.3
PsSW 17 (17) (Clade IV)	-5.1	1.9

Fig S4B: Line 1/55-AAP1(3a) vs 'BOHATYR' (Seed Coats)

Target Genes	Developmental stages						
	12d	14d	16d	18d	20d	23d	30d
PsIPT 1 (604)	1.3	1.1	-1.7	-6.9	-5.9	7.3	-2.6
PsIPT 2 (605)	4.0	1.2	1.8	-1.0	-2.2	-2.8	-1.9
PsIPT 4 (421)	7.7	5.0	16.5	3.8	5.1	4.1	3.9
PsCKX 1 (930)	2.7	2.5	3.0	3.5	2.6	2.8	2.0
PsCKX 2 (627)	11.7	6.5	14.8	8.9	19.8	3.8	5.6
PsCKX 5 (942)	-	-	-	-	-	-	-
PsCKX 7 (910)	1.4	1.1	2.0	1.2	1.3	1.3	1.0
PsSUT 1 (366)	2.0	1.7	1.5	6.3	6.5	1.1	-1.1
PsSUT 2 (948)	30.4	14.3	15.6	17.4	6.8	4.6	3.8
PsSUT 3 (674)	1.4	1.1	6.0	3.8	3.7	1.6	1.3
PsSUT 5 (666)	1.2	10.3	8.0	3.6	2.8	2.2	2.2
PsAAP 1(3a) (532 Transgene)	19.3	90.5	109.5	113.6	353.1	16.9	23.9
PsAAP 7a (498)(Cluster 1)	2.6	2.4	3.8	6.3	26.1	2.5	14.3
PsAAP 7b (9261)	3.0	1.2	-2.2	7.8	-3.0	1.6	2.6
PsAAP 2a (675)(Cluster 3A)	6.4	14.4	53.2	55.5	80.1	10.5	7.1
PsAAP 2c (4401)	1.4	2.2	2.0	1.2	1.3	1.3	1.0
PsAAP 2d (840)	1.8	2.9	5.9	1.0	1.3	-4.2	-1.4
PsAAP 3b (051)	1.1	3.5	11.7	17.9	13.9	3.3	2.1
PsAAP 6a (931)(Cluster 4B)	1.6	-1.5	2.3	1.2	1.1	1.6	-21.3
PsAAP 6b (328)	1.6	2.2	5.1	1.4	6.7	1.3	-1.3
PsAAP 1 (180)	-1.3	-1.0	2.6	4.2	3.3	-1.3	1.5
PsCWINV 1 (240)	1.6	2.0	1.2	2.7	4.1	1.1	10.1
PsCWINV 2 (448)	1.1	2.2	2.8	11.9	25.5	1.5	14.1
PsCWINV 3 (415)	3.0	3.3	6.3	9.0	8.2	2.6	2.4
PsCWINV 6 (320)	2.3	1.7	2.7	2.4	1.6	1.3	1.5
PsSW 2a	2.7	3.7	-3.2	5.0	-2.1	-1.6	1.0
PsSW 13	3.0	1.2	-2.2	7.8	-3.0	1.6	2.6
PsSW 12 (13b)	1.2	2.5	-1.5	2.7	-2.3	1.8	1.7

Fig S4C: Line 1/55-AAP1(3a) vs 'BOHATYR' (Cotyledons)

Target Genes	Developmental stages						
	12d	14d	16d	18d	20d	23d	30d
PsIPT 1 (604)	2.0	5.9	1.1	1.9	1.1	2.9	
PsIPT 2 (605)	1.8	6.1	-2.2	2.3	1.1	2.5	
PsIPT 4 (421)	7.7	5.0	16.5	3.8	5.1	4.1	3.9
PsCKX 1 (930)	9.3	-1.4	-3.6	3.2	-2.5	1.5	7.3
PsCKX 2 (627)	1.1	1.8	-4.5	1.5	-2.9	1.2	2.1
PsCKX 5 (942)	2.0	1.3	1.1	1.2	1.1	1.2	1.4
PsCKX 7 (910)	3.6	1.6	-3.6	2.0	-2.0	-1.0	1.9
PsSUT 1 (366)	2.3	1.5	1.0	3.8	-2.4	-1.4	1.1
PsSUT 2 (948)	1.6	1.3	1.2	2.6	1.3	1.2	3.2
PsSUT 3 (674)	-7.0	1.2	1.4	-2.7	-6.7	-4.7	-1.0
PsSUT 5 (666)	8.4	2.9	1.3	2.1	4.1	2.4	2.7
PsAAP 1(3a) (532 Transgene)	-1.6	2.9	-1.6	1.1	1.1	2.7	-1.9
PsAAP 7a (498)(Cluster 1)	-1.6	2.6	-2.3	7.4	-1.5	1.1	4.1
PsAAP 7b (9261)	-1.2	-0.4	-1.1	1.2	1.6	2.0	-2.6
PsAAP 2a (675)(Cluster 3A)	-1.6	-1.2	-2.4	-1.9	-6.8	-2.0	1.3
PsAAP 2c (4401)	2.1	1.9	-2.3	1.7	-1.1	1.0	6.0
PsAAP 2d (840)	3.1	1.4	-2.6	1.3	1.1	1.8	3.2
PsAAP 3b (051)	1.7	4.3	-29.5	-1.3	-10.3	-1.1	1.4
PsAAP 6a (931) (Cluster 4B)	2.8	1.6	-2.7	1.3	-2.1	1.6	2.8
PsAAP 6b (328)	-2.7	1.4	-3.2	2.9	1.6	6.4	10.3
PsAAP 1 (180)	4.7	1.4	-2.7	-1.2	1.2	-1.0	3.9
PsCWINV 1 (240)	-2.2	3.9	-9.4	7.8	-8.6	6.2	-4.4
PsCWINV 2 (448)	-21.6	1.0	-16.7	1.4	2.6	1.5	-2.0
PsCWINV 3 (415)	-1.5	1.8	-3.5	4.4	-1.7	-1.8	-8.6
PsCWINV 6 (320)	1.2	3.5	-7.3	2.5	-1.1	13.0	-1.4
PsSW 2a	2.7	3.7	-3.2	5.0	-2.1	-1.6	1.0
PsSW 13	3.0	1.2	-2.2	7.8	-3.0	1.6	2.6
PsSW 12 (13b)	1.2	2.5	-1.5	2.7	-2.3	1.8	1.7

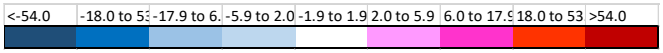


Fig. S4. Relative gene expression of cytokinin biosynthesis (*PsIPT*), cytokinin degradation (*PsCKX*), sucrose transporters (*PsSUT*), amino acid transporters (*PsAAP*), cell wall invertase (*PsINV*) and *PsSWEET* (*PsSW*) gene family members in

- **A)** Leaves of transgenic line 1/55-AAP1(3a) relative to 'Bohatyr' from 5 d to 20 d (d=Days)
- **B)** Seed coats of transgenic line 1/55-AAP1(3a) relative to 'Bohatyr' from 12-30 DAP
- **C)** Cotyledons of transgenic line 1/55-AAP1(3a) relative to 'Bohatyr' from 12-30 DAP

Values are fold-changes relative to the expression in the wildtype 'Bohatyr' line. The colour scale indicates up-regulated expression (red), similar expression (white), and down-regulated expression (blue) relative to that in the wildtype line.

Table S1. Cytokinin analysis for pods, podwalls, seedcoats and cotyledons for wildtype 'Bolero' and the double transgenic line 562 for total cytokinins, tZ-types, cZ-types, DHZ-types, and total iP-types. Cytokinin levels are in pmol/g dry weight (Mean ± SD; RSD=Relative Standard Deviation)

Sample	*Total Cytokinins*			*Total CK Bases*			*Total CK Ribosides*			*Total CK Nucleotides*			*Total CK O-glucosides*			*Total CK N-glucosides*		
Bolero 1 DAF pod	**266.22**	±	**31.29**	103.78	±	3.63	26.70	±	3.65	97.93	±	18.80	31.74	±	5.32	6.07	±	0.63
Bolero 3 DAF pod	**147.42**	±	**25.90**	59.40	±	6.74	13.04	±	3.02	63.43	±	16.05	9.53	±	1.34	2.01	±	0.43
562 0 DAF pod	**189.51**			108.09			35.43			27.02			18.29	0.00		0.69		
562 1 DAF pod	**160.19**	±	**11.06**	72.12	±	4.40	10.48	±	0.37	54.41	±	1.63	21.97	±	4.35	1.22	±	0.31
Bolero 7 DAF Podwall	**189.90**	±	**4.95**	67.82	±	7.28	5.21	±	0.32	91.83	±	15.59	23.68	±	3.20	1.35	±	0.30
Bolero 10 DAF Podwall	**197.42**	±	**2.81**	43.11	±	2.97	11.76	±	1.09	115.49	±	4.72	26.17	±	1.18	0.89	±	0.10
Bolero 12 DAF Podwall	**171.85**	±	**30.16**	29.79	±	3.89	9.15	±	1.23	105.88	±	19.57	26.25	±	5.73	0.78	±	0.14
Bolero 14 DAF Podwall	**147.07**	±	**9.65**	32.63	±	0.80	8.84	±	1.81	80.72	±	8.68	24.60	±	5.07	0.28	±	0.04
Bolero 16 DAF Podwall	**141.23**	±	**5.10**	30.85	±	2.12	10.95	±	1.28	77.23	±	3.73	22.03	±	2.10	0.16	±	0.02
562 7 DAF Podwall	**166.01**	±	**7.90**	70.18	±	2.38	3.29	±	0.47	68.01	±	9.18	23.61	±	0.42	0.93	±	0.16
562 10 DAF Podwall	**221.76**	±	**33.87**	63.03	±	4.30	8.44	±	1.41	123.94	±	27.23	25.91	±	6.38	0.44	±	0.07
562 14 DAF Podwall	**183.37**	±	**29.88**	28.07	±	0.25	15.20	±	2.86	105.38	±	20.58	34.39	±	6.64	0.33	±	0.06
562 16 DAF Podwall	**143.64**	±	**18.73**	23.23	±	2.97	11.94	±	1.42	79.51	±	13.65	28.67	±	1.08	0.30	±	0.03
562 20 DAF Podwall	**120.53**	±	**9.32**	37.49	±	6.54	5.08	±	0.33	39.29	±	1.35	38.22	±	5.48	0.46	±	0.05
Bolero 10 DAF Seedcoa	**370.66**	±	**56.10**	50.30	±	11.86	19.93	±	2.36	276.28	±	41.29	14.36	±	2.51	9.78	±	1.92
Bolero 12 DAF Seedcoa	**188.63**	±	**35.93**	32.20	±	5.40	8.94	±	0.68	138.20	±	31.12	7.04	±	1.35	2.25	±	0.64
Bolero 14 DAF Seedcoa	**1000.04**	±	**59.62**	37.38	±	6.45	39.92	±	6.75	911.55	±	47.39	9.85	±	1.06	1.34	±	0.10
Bolero 16 DAF Seedcoa	**144.35**	±	**12.19**	22.80	±	3.24	7.43	±	0.57	104.94	±	15.82	7.09	±	1.46	2.09	±	0.54
Bolero 20 DAF Seedcoa	**180.25**	±	**35.80**	22.16	±	5.09	10.01	±	0.97	137.63	±	28.72	8.52	±	2.06	1.92	±	0.21
562 10 DAF Seedcoat	**150.70**	±	**32.87**	20.94	±	2.99	11.12	±	2.72	104.73	±	24.48	6.04	±	0.84	7.88	±	2.27
562 12 DAF Seedcoat	**492.80**	±	**115.76**	25.47	±	5.28	12.04	±	2.75	444.09	±	108.86	8.23	±	1.07	2.96	±	0.47
562 14 DAF Seedcoat	**921.87**	±	**108.78**	43.86	±	7.32	30.40	±	1.74	837.77	±	104.18	8.17	±	1.26	1.68	±	0.36
562 16 DAF Seedcoat	**1139.11**	±	**217.62**	27.68	±	2.62	42.69	±	10.80	1058.28	±	221.46	9.60	±	1.39	0.86	±	0.17
562 20 DAF Seedcoat	**957.35**	±	**221.68**	30.07	±	6.75	27.04	±	5.40	882.98	±	208.87	15.83	±	4.07	1.43	±	0.35
Bolero 7 DAF Cotyledo	**162.81**	±	**28.31**	53.82	±	6.33	10.65	±	1.77	79.75	±	16.43	4.77	±	0.42	13.82	±	3.36
Bolero 10 DAF Cotyled	**239.16**	±	**50.47**	30.59	±	3.85	21.01	±	1.89	173.99	±	44.49	10.38	±	0.63	3.20	±	0.37
Bolero 12 DAF Cotyled	**34.45**			20.74	0.00		12.16			0.00			1.41			0.15	0.00	0.00
Bolero 14 DAF Cotyled	**74.71**	±	**14.71**	20.65	±	4.42	4.17	±	0.90	43.64	±	10.15	6.18	±	1.61	0.07	±	0.01
Bolero 16 DAF Cotyled	**47.21**	±	**6.32**	16.47	±	1.17	2.22	±	0.16	24.14	±	4.61	4.35	±	0.65	0.03	±	0.01
Bolero 20 DAF Cotyled	**148.09**	±	**17.30**	39.93	±	0.85	4.66	±	0.50	99.69	±	19.03	3.79	±	0.38	0.02	±	0.00
562 7 DAF Cotyledon	**198.99**	±	**44.57**	31.18	±	4.38	12.59	±	0.45	131.29	±	32.52	6.47	±	0.08	17.47	±	7.30
562 12 DAF Cotyledon	**107.30**			26.65			10.44			63.85			6.32			0.05		
562 14 DAF Cotyledon	**87.88**	±	**18.46**	14.55	±	3.26	2.17	±	0.31	67.21	±	17.77	3.92	±	0.45	0.03		
562 16 DAF Cotyledon	**80.28**	±	**2.83**	23.32	±	3.86	2.42	±	0.55	49.13	±	1.26	5.35	±	1.09	0.06	±	0.01
562 20 DAF Cotyledon	**39.46**	±	**2.03**	13.83	±	3.25	1.63	±	0.04	20.93	±	4.50	3.04	±	0.11	0.02	±	0.00

Sample	*Total tZ-types*			*tZ*			*tZR*			*tZRMP*			*tZOG*			*tZROG*			*tZ7G*			*tZ9G*
Bolero 1 DAF pod	**29.90**	±	**4.20**	19.38	±	3.19	0.97	±	0.06	5.08	±	0.53	4.88	±	0.71	0.25	±	0.05	1.02	±	0.15	*<LOD*
Bolero 3 DAF pod	**24.74**	±	**3.68**	12.53	±	2.43	1.33	±	0.18	7.64	±	1.04	2.21	±	0.54	0.49	±	0.08	0.55	±	0.13	*<LOD*
562 0 DAF pod	**27.27**			13.77			0.65			8.33			3.98	0.00		0.24			0.30			*<LOD*
562 1 DAF pod	**19.12**	±	**3.12**	9.65	±	1.23	0.51	±	0.05	4.29	±	0.61	4.26	±	1.17	0.14	±	0.03	0.28	±	0.03	*<LOD*
Bolero 7 DAF Podwall	**33.00**	±	**2.69**	12.43	±	2.41	0.40	±	0.01	12.29	±	1.71	6.16	±	0.19	1.05	±	0.12	0.67	±	0.10	*<LOD*
Bolero 10 DAF Podwall	**36.91**	±	**3.43**	5.18	±	0.76	3.10	±	0.09	20.87	±	4.18	4.84	±	0.18	2.52	±	0.07	0.40	±	0.04	*<LOD*
Bolero 12 DAF Podwall	**30.25**	±	**5.59**	4.87	±	1.05	1.87	±	0.41	14.46	±	2.46	5.75	±	1.04	2.77	±	0.72	0.52	±	0.09	*<LOD*
Bolero 14 DAF Podwall	**20.06**	±	**3.10**	2.24	±	0.40	1.18	±	0.25	8.32	±	1.03	5.12	±	1.20	3.04	±	0.56	0.16	±	0.03	*<LOD*
Bolero 16 DAF Podwall	**23.62**	±	**1.20**	2.92	±	0.51	2.94	±	0.21	9.78	±	0.27	4.99	±	0.78	2.92	±	0.05	0.06	±	0.01	*<LOD*
562 7 DAF Podwall	**31.99**	±	**1.98**	12.84	±	1.23	0.38	±	0.10	11.98	±	2.06	5.74	±	0.48	0.65	±	0.02	0.39	±	0.04	*<LOD*
562 10 DAF Podwall	**38.63**	±	**7.14**	7.85	±	1.33	1.58	±	0.19	22.47	±	4.69	4.06	±	0.80	2.44	±	0.49	0.22	±	0.04	*<LOD*
562 14 DAF Podwall	**26.02**	±	**4.23**	2.45	±	0.36	3.17	±	0.41	12.53	±	1.82	4.23	±	0.87	3.51	±	0.75	0.13	±	0.02	*<LOD*
562 16 DAF Podwall	**20.11**	±	**1.23**	2.71	±	0.39	2.35	±	0.17	7.48	±	0.62	4.22	±	0.06	3.23	±	0.08	0.12	±	0.01	*<LOD*
562 20 DAF Podwall	**12.53**	±	**1.06**	1.92	±	0.27	0.27	±	0.05	1.61	±	0.27	4.23	±	0.65	4.87	±	1.17	0.17	±	0.01	*<LOD*
Bolero 10 DAF Seedcoa	**11.83**	±	**0.15**	7.16	±	1.04	0.27	±	0.05	3.40	±	1.02	0.59	±	0.07	0.31	±	0.01	0.10	±	0.02	*<LOD*
Bolero 12 DAF Seedcoa	**6.05**	±	**0.85**	4.60	±	0.41	0.13	±	0.01	1.57	±	0.50	0.20	±	0.05	0.09	±	0.02	0.06	±	0.01	*<LOD*
Bolero 14 DAF Seedcoa	**20.46**	±	**1.14**	3.96	±	0.32	0.59	±	0.02	15.01	±	0.84	0.48	±	0.02	0.35	±	0.03	0.07	±	0.01	*<LOD*
Bolero 16 DAF Seedcoa	**4.50**	±	**0.86**	2.59	±	0.51	0.13	±	0.01	2.03	±	0.14	0.22	±	0.03	0.16	±	0.02	0.05	±	0.01	*<LOD*
Bolero 20 DAF Seedcoa	**5.74**	±	**1.12**	3.65	±	0.78	0.17	±	0.03	1.39	±	0.26	0.30	±	0.06	0.19	±	0.04	0.04	±	0.00	*<LOD*
562 10 DAF Seedcoat	**4.21**	±	**0.94**	3.59	±	0.71	0.10	±	0.02		*<LOD*		0.44	±	0.12	0.16	±	0.04	0.06	±	0.01	*<LOD*
562 12 DAF Seedcoat	**18.70**	±	**3.39**	4.82	±	1.17	0.35	±	0.07	13.28	±	2.33	0.21	±	0.01	0.09	±	0.00	0.02	±	0.01	*<LOD*
562 14 DAF Seedcoat	**25.46**	±	**4.13**	6.56	±	1.49	0.62	±	0.03	17.86	±	2.62	0.27	±	0.03	0.11	±	0.00	0.04	±	0.01	*<LOD*
562 16 DAF Seedcoat	**11.28**	±	**2.89**	3.47	±	0.78	0.23	±	0.04	7.02	±	2.14	0.33	±	0.03	0.20	±	0.03	0.04	±	0.01	*<LOD*
562 20 DAF Seedcoat	**4.66**	±	**1.49**	1.60	±	0.29	0.12	±	0.02	2.90	±	0.39	0.56	±	0.09	0.40	±	0.07	0.05	±	0.01	*<LOD*
Bolero 7 DAF Cotyledo	**7.29**	±	**0.43**	5.98	±	0.34	0.22	±	0.03		*<LOD*		0.82	±	0.12	0.13	±	0.02	0.15	±	0.02	*<LOD*
Bolero 10 DAF Cotyled	**10.48**	±	**1.42**	4.66	±	0.37	0.19	±	0.04	5.09	±	1.54	0.35	±	0.04	0.13	±	0.03	0.05	±	0.01	*<LOD*
Bolero 12 DAF Cotyled	**4.08**	**0.00**		3.45	0.00		0.48				*<LOD*			*<LOD*			*<LOD*		0.15			*<LOD*
Bolero 14 DAF Cotyled	**5.22**	±	**0.81**	4.59	±	1.02	0.16	±	0.03	0.61	±	0.00	0.20	±	0.05		*<LOD*		0.07	±	0.01	*<LOD*
Bolero 16 DAF Cotyled	**4.26**	±	**1.41**	3.52	±	0.74	0.06	±	0.01	1.60	±	0.00	0.15	±	0.01	0.04	±	0.01	0.03	±	0.01	*<LOD*
Bolero 20 DAF Cotyled	**2.16**	±	**0.14**	1.86	±	0.12	0.07	±	0.02		*<LOD*		0.09	±	0.03	0.11	±	0.03	0.02	±	0.00	*<LOD*
562 7 DAF Cotyledon	**6.20**	±	**0.78**	4.94	±	0.58	0.19	±	0.03		*<LOD*		0.79	±	0.18	0.16	±	0.03	0.12	±	0.02	*<LOD*
562 12 DAF Cotyledon	**5.60**			4.28			0.21			0.49			0.57				*<LOD*		0.05			*<LOD*
562 14 DAF Cotyledon	**4.44**	±	**0.64**	3.36	±	0.56	0.07	±	0.01	1.22	±	0.01	0.16	±	0.03		*<LOD*		0.03	±	0.00	*<LOD*
562 16 DAF Cotyledon	**4.48**	±	**0.36**	3.86	±	0.80	0.09	±	0.01	1.04	±	0.00	0.13	±	0.00	0.07	±	0.02	0.06	±	0.01	*<LOD*
562 20 DAF Cotyledon	**1.52**	±	**0.07**	1.27	±	0.11	0.07	±	0.01		*<LOD*		0.07	±	0.01	0.11	±	0.01	0.02	±	0.00	*<LOD*

Sample	*Total cZ-types*			*cZ*			*cZR*			*cZRMP*			*cZOG*			*cZROG*			*cZ7G*			*cZ9G*
Bolero 1 DAF pod	**132.40**	±	**23.82**	21.77	±	3.55	14.70	±	1.13	79.40	±	17.40	7.33	±	0.98	9.21	±	1.87		*<LOD*		*<LOD*
Bolero 3 DAF pod	**58.09**	±	**15.52**	14.85	±	4.24	2.59	±	0.66	37.65	±	10.37	2.61	±	0.45	0.39	±	0.09		*<LOD*		*<LOD*
562 0 DAF pod	**16.77**			9.30			2.52			<LOD			4.67	0.00		0.28				*<LOD*		*<LOD*
562 1 DAF pod	**43.66**	±	**2.90**	8.42	±	2.46	1.69	±	0.19	28.85	±	4.94	4.36	±	0.32	0.35	±	0.10		*<LOD*		*<LOD*
Bolero 7 DAF Podwall	**115.10**	±	**11.21**	31.64	±	1.53	1.75	±	0.35	72.54	±	14.85	8.38	±	1.78	0.79	±	0.16		*<LOD*		*<LOD*
Bolero 10 DAF Podwall	**84.25**	±	**7.02**	2.84	±	0.36	1.98	±	0.18	70.77	±	7.00	6.11	±	0.19	2.57	±	0.27		*<LOD*		*<LOD*
Bolero 12 DAF Podwall	**94.23**	±	**16.45**	4.86	±	0.71	2.87	±	0.53	78.63	±	14.95	6.25	±	1.15	1.63	±	0.40		*<LOD*		*<LOD*
Bolero 14 DAF Podwall	**74.13**	±	**8.26**	6.82	±	1.22	2.47	±	0.58	58.03	±	10.32	5.31	±	1.39	1.50	±	0.24		*<LOD*		*<LOD*
Bolero 16 DAF Podwall	**71.25**	±	**2.49**	4.36	±	0.79	2.45	±	0.16	57.87	±	3.71	5.21	±	1.37	1.35	±	0.06		*<LOD*		*<LOD*
562 7 DAF Podwall	**65.44**	±	**3.19**	18.45	±	2.42	0.66	±	0.06	39.20	±	5.43	6.70	±	0.05	0.43	±	0.05		*<LOD*		*<LOD*
562 10 DAF Podwall	**104.11**	±	**18.21**	12.13	±	2.66	1.83	±	0.47	83.49	±	18.08	4.71	±	0.98	1.96	±	0.53		*<LOD*		*<LOD*
562 14 DAF Podwall	**86.36**	±	**16.67**	2.24	±	0.67	4.05	±	0.85	72.09	±	15.06	5.45	±	1.09	2.53	±	0.34		*<LOD*		*<LOD*
562 16 DAF Podwall	**62.39**	±	**9.96**	3.31	±	0.80	2.07	±	0.32	49.15	±	9.64	5.10	±	0.28	2.76	±	0.33		*<LOD*		*<LOD*
562 20 DAF Podwall	**51.15**	±	**4.42**	8.36	±	2.45	1.59	±	0.22	32.11	±	1.01	6.12	±	0.88	2.96	±	0.41		*<LOD*		*<LOD*
Bolero 10 DAF Seedcoa	**180.45**	±	**43.09**	14.76	±	4.43	6.32	±	1.56	150.22	±	34.59	8.29	±	2.50	0.86	±	0.01		*<LOD*		*<LOD*
Bolero 12 DAF Seedcoa	**74.38**	±	**15.75**	6.94	±	1.61	3.50	±	0.52	58.80	±	15.09	4.49	±	0.84	0.64	±	0.12		*<LOD*		*<LOD*
Bolero 14 DAF Seedcoa	**193.20**	±	**30.18**	4.82	±	1.40	4.15	±	0.17	176.72	±	29.93	6.89	±	1.02	0.62	±	0.03		*<LOD*		*<LOD*
Bolero 16 DAF Seedcoa	**50.71**	±	**5.00**	3.39	±	0.87	2.13	±	0.48	40.39	±	6.75	4.42	±	1.21	0.37	±	0.03		*<LOD*		*<LOD*
Bolero 20 DAF Seedcoa	**82.57**	±	**20.47**	5.96	±	1.49	3.55	±	0.71	65.80	±	17.82	6.69	±	1.78	0.58	±	0.04		*<LOD*		*<LOD*
562 10 DAF Seedcoat	**80.64**	±	**17.58**	3.48	±	0.25	5.00	±	1.35	69.76	±	16.20	1.91	±	0.33	0.50	±	0.10		*<LOD*		*<LOD*
562 12 DAF Seedcoat	**118.03**	±	**19.78**	3.98	±	1.13	2.53	±	0.21	106.06	±	17.94	4.73	±	0.78	0.72	±	0.14		*<LOD*		*<LOD*
562 14 DAF Seedcoat	**206.69**	±	**20.95**	4.97	±	0.91	7.02	±	1.29	188.00	±	20.46	5.98	±	1.23	0.71	±	0.10		*<LOD*		*<LOD*
562 16 DAF Seedcoat	**90.91**	±	**23.72**	2.20	±	0.59	1.87	±	0.35	78.66	±	21.74	7.52	±	1.24	0.65	±	0.10		*<LOD*		*<LOD*
562 20 DAF Seedcoat	**121.50**	±	**25.73**	4.58	±	0.99	1.44	±	0.12	101.78	±	22.12	13.09	±	3.66	0.61	±	0.13		*<LOD*		*<LOD*
Bolero 7 DAF Cotyledo	**49.01**	±	**12.31**	3.29	±	0.81	2.93	±	0.86	41.16	±	10.77	1.08	±	0.23	0.54	±	0.10		*<LOD*		*<LOD*
Bolero 10 DAF Cotyled	**101.91**	±	**23.72**	2.85	±	0.41	8.20	±	1.21	86.15	±	23.86	3.63	±	0.95	1.08	±	0.19		*<LOD*		*<LOD*
Bolero 12 DAF Cotyled	**5.14**	**0.00**		0.79	0.00		3.43			<LOD			0.92			<LOD	0.00	0.00		*<LOD*		*<LOD*
Bolero 14 DAF Cotyled	**31.85**	±	**6.19**	1.25	±	0.25	1.06	±	0.25	23.88	±	5.73	5.57	±	1.54	0.13	±	0.03		*<LOD*		*<LOD*
Bolero 16 DAF Cotyled	**21.98**	±	**4.53**	1.89	±	0.39	0.50	±	0.12	15.59	±	3.87	3.86	±	0.65	0.14	±	0.04		*<LOD*		*<LOD*
Bolero 20 DAF Cotyled	**104.18**	±	**19.17**	3.08	±	0.53	2.25	±	0.06	95.39	±	18.96	3.38	±	0.38	0.08	±	0.00		*<LOD*		*<LOD*
562 7 DAF Cotyledon	**89.42**	±	**25.07**	3.16	±	0.65	4.23	±	1.01	77.38	±	23.93	4.17	±	0.64	0.47	±	0.12		*<LOD*		*<LOD*
562 12 DAF Cotyledon	**61.15**			2.11			4.77			48.80			5.47			<LOD				*<LOD*		*<LOD*
562 14 DAF Cotyledon	**54.16**	±	**13.15**	3.76	±	1.06	0.86	±	0.10	45.95	±	13.34	3.56	±	0.40	0.05				*<LOD*		*<LOD*
562 16 DAF Cotyledon	**37.15**	±	**2.11**	1.69	±	0.48	0.67	±	0.12	29.81	±	1.21	4.86	±	1.06	0.12	±	0.03		*<LOD*		*<LOD*
562 20 DAF Cotyledon	**24.06**	±	**4.46**	1.32	±	0.41	0.82	±	0.18	19.14	±	4.58	2.72	±	0.15	0.06	±	0.02		*<LOD*		*<LOD*

Sample	*Total DHZ-types*			*DHZ*			*DHZR*			*DHZRMP*			*DHZOG*			*DHZROG*			*DHZ7G*			*DHZ9G*		
Bolero 1 DAF pod	**55.13**	±	**11.24**	27.45	±	6.36	7.31	±	1.55	5.25	±	1.34	8.36	±	1.98	1.71	±	0.41	5.05	±	0.52		*<LOD*	
Bolero 3 DAF pod	**22.94**	±	**5.31**	7.80	±	2.23	5.03	±	1.42	4.81	±	1.26	3.42	±	0.80	0.62	±	0.17	1.47	±	0.34		*<LOD*	
562 0 DAF pod	**69.25**			41.59			5.45			12.72			9.11	0.00			*<LOD*		0.39				*<LOD*	
562 1 DAF pod	**56.98**	±	**13.92**	28.15	±	6.58	5.28	±	0.92	9.75	±	2.77	12.24	±	3.26	0.63	±	0.12	0.94	±	0.27		*<LOD*	
Bolero 7 DAF Podwall	**25.93**	±	**2.72**	15.19	±	1.94	1.67	±	0.42	1.09	±	0.27	6.30	±	0.98	1.00	±	0.09	0.61	±	0.20		*<LOD*	
Bolero 10 DAF Podwall	**44.20**	±	**1.43**	24.55	±	0.95	4.14	±	0.58	4.89	±	0.21	8.78	±	0.33	1.35	±	0.20	0.40	±	0.07	0.10	±	0.02
Bolero 12 DAF Podwall	**26.85**	±	**6.18**	12.47	±	3.22	2.41	±	0.20	1.86	±	0.41	8.96	±	2.24	0.89	±	0.21	0.18	±	0.05	0.08	±	0.02
Bolero 14 DAF Podwall	**22.85**	±	**4.29**	8.40	±	1.32	2.69	±	0.74	2.01	±	0.61	8.73	±	1.59	0.90	±	0.17	0.05	±	0.00	0.07	±	0.01
Bolero 16 DAF Podwall	**20.52**	±	**1.39**	7.17	±	0.42	3.74	±	0.63	1.95	±	0.41	6.28	±	0.34	1.27	±	0.16	0.04	±	0.01	0.06	±	0.00
562 7 DAF Podwall	**31.64**	±	**0.77**	18.64	±	0.73	0.90	±	0.14	1.47	±	0.18	9.54	±	0.25	0.54	±	0.04	0.49	±	0.12	0.06	±	0.01
562 10 DAF Podwall	**40.29**	±	**7.60**	19.73	±	3.93	3.69	±	0.52	3.91	±	1.20	11.57	±	3.26	1.17	±	0.37	0.15	±	0.03	0.07	±	0.01
562 14 DAF Podwall	**37.67**	±	**7.16**	11.14	±	2.38	4.61	±	0.92	3.05	±	0.24	16.72	±	3.13	1.95	±	0.46	0.10	±	0.02	0.11	±	0.01
562 16 DAF Podwall	**25.85**	±	**1.95**	7.42	±	0.43	3.40	±	0.85	1.49	±	0.10	11.93	±	0.56	1.42	±	0.17	0.07	±	0.01	0.11	±	0.02
562 20 DAF Podwall	**27.48**	±	**4.19**	5.11	±	0.56	1.73	±	0.42	0.49	±	0.06	17.70	±	2.81	2.33	±	0.59	0.12	±	0.03	0.18	±	0.03
Bolero 10 DAF Seedcoa	**40.62**	±	**1.90**	9.02	±	1.03	3.98	±	0.51	13.63	±	0.41	1.00	±	0.09	3.31	±	0.15	9.68	±	1.94		*<LOD*	
Bolero 12 DAF Seedcoa	**13.64**	±	**1.93**	4.68	±	1.08	1.41	±	0.44	3.68	±	0.82	0.44	±	0.09	1.24	±	0.33	2.19	±	0.63		*<LOD*	
Bolero 14 DAF Seedcoa	**18.51**	±	**0.27**	3.98	±	0.41	2.41	±	0.54	9.33	±	0.17	0.54	±	0.03	0.98	±	0.04	1.27	±	0.11		*<LOD*	
Bolero 16 DAF Seedcoa	**14.76**	±	**3.27**	3.26	±	0.87	1.66	±	0.46	5.89	±	1.21	0.42	±	0.11	1.50	±	0.25	2.03	±	0.53		*<LOD*	
Bolero 20 DAF Seedcoa	**8.43**	±	**1.02**	1.41	±	0.22	1.61	±	0.49	2.76	±	0.64	0.23	±	0.05	0.54	±	0.14	1.70	±	0.21	0.18	±	0.02
562 10 DAF Seedcoat	**31.51**	±	**7.59**	4.81	±	0.61	2.79	±	0.69	12.91	±	3.59	0.63	±	0.13	2.55	±	0.54	7.82	±	2.26		*<LOD*	
562 12 DAF Seedcoat	**24.24**	±	**3.52**	5.46	±	0.68	1.76	±	0.51	11.53	±	2.36	0.51	±	0.11	2.05	±	0.16	2.94	±	0.47		*<LOD*	
562 14 DAF Seedcoat	**14.78**	±	**0.71**	3.69	±	0.10	1.48	±	0.21	6.88	±	0.93	0.35	±	0.02	0.74	±	0.07	1.64	±	0.36		*<LOD*	
562 16 DAF Seedcoat	**10.10**	±	**2.09**	2.94	±	0.72	0.79	±	0.16	4.64	±	1.34	0.34	±	0.06	0.57	±	0.06	0.72	±	0.20	0.10	±	0.03
562 20 DAF Seedcoat	**9.43**	±	**1.76**	2.01	±	0.43	0.80	±	0.17	4.06	±	0.63	0.58	±	0.16	0.60	±	0.14	1.10	±	0.29	0.28	±	0.06
Bolero 7 DAF Cotyledo	**25.28**	±	**3.72**	3.73	±	0.26	1.83	±	0.37	3.84	±	0.56	0.86	±	0.09	1.35	±	0.36	13.67	±	3.34		*<LOD*	
Bolero 10 DAF Cotyled	**25.12**	±	**3.53**	6.89	±	0.44	1.58	±	0.34	8.32	±	2.16	0.71	±	0.05	4.47	±	1.08	3.15	±	0.37		*<LOD*	
Bolero 12 DAF Cotyled	**6.60**	**0.00**		0.83	0.00		5.29				*<LOD*		0.48				*<LOD*			*<LOD*			*<LOD*	
Bolero 14 DAF Cotyled	**1.77**	±	**0.46**	0.59	±	0.15	0.87	±	0.25		*<LOD*		0.31	±	0.07		*<LOD*			*<LOD*			*<LOD*	
Bolero 16 DAF Cotyled	**1.31**	±	**0.20**	0.36	±	0.01	0.73	±	0.20		*<LOD*		0.22	±	0.01		*<LOD*			*<LOD*			*<LOD*	
Bolero 20 DAF Cotyled	**1.14**	±	**0.76**	0.23	±	0.10	0.78	±	0.65		*<LOD*		0.13	±	0.00		*<LOD*			*<LOD*			*<LOD*	
562 7 DAF Cotyledon	**32.81**	±	**9.33**	4.56	±	1.21	3.98	±	0.83	6.05	±	1.45	0.88	±	0.23		*<LOD*		17.34	±	7.27		*<LOD*	
562 12 DAF Cotyledon	**6.06**			1.32			3.31			1.16			0.27				*<LOD*			*<LOD*			*<LOD*	
562 14 DAF Cotyledon	**1.74**	±	**0.31**	0.35	±	0.11	0.50	±	0.13	0.73	±	0.20	0.16	±	0.03		*<LOD*			*<LOD*			*<LOD*	
562 16 DAF Cotyledon	**1.01**	±	**0.25**	0.30	±	0.07	0.47	±	0.14		*<LOD*		0.23	±	0.04		*<LOD*			*<LOD*			*<LOD*	
562 20 DAF Cotyledon	**0.64**	±	**0.10**	0.21	±	0.04	0.32	±	0.08		*<LOD*		0.10	±	0.01		*<LOD*			*<LOD*			*<LOD*	

Sample	*Total iP-types*			*iP*			*iPR*			*iPRMP*			*iP7G*			*iP9G*	
Bolero 1 DAF pod	**48.79**	±	**7.39**	35.17	±	9.05	3.72	±	0.96	9.90	±	1.79	*<LOD*			*<LOD*	
Bolero 3 DAF pod	**41.65**	±	**1.59**	24.21	±	2.68	4.10	±	1.19	13.33	±	3.54	*<LOD*			*<LOD*	
562 0 DAF pod	**76.22**			43.43			26.81			5.97			*<LOD*			*<LOD*	
562 1 DAF pod	**40.43**	±	**3.08**	25.90	±	5.87	3.00	±	0.40	11.53	±	3.19	*<LOD*			*<LOD*	
Bolero 7 DAF Podwall	**15.87**	±	**1.10**	8.58	±	1.78	1.38	±	0.13	5.91	±	0.87	*<LOD*			*<LOD*	
Bolero 10 DAF Podwal	**32.06**	±	**1.22**	10.54	±	1.47	2.55	±	0.31	18.97	±	0.18	*<LOD*			*<LOD*	
Bolero 12 DAF Podwal	**20.52**	±	**2.49**	7.59	±	1.52	2.00	±	0.36	10.93	±	2.18	*<LOD*			*<LOD*	
Bolero 14 DAF Podwal	**30.02**	±	**0.78**	15.17	±	3.46	2.50	±	0.47	12.35	±	2.88	*<LOD*			*<LOD*	
Bolero 16 DAF Podwal	**25.85**	±	**3.91**	16.39	±	2.94	1.83	±	0.30	7.63	±	1.02	*<LOD*			*<LOD*	
562 7 DAF Podwall	**36.93**	±	**3.93**	20.24	±	1.99	1.34	±	0.28	15.35	±	2.11	*<LOD*			*<LOD*	
562 10 DAF Podwall	**38.74**	±	**6.34**	23.32	±	6.54	1.34	±	0.39	14.07	±	3.84	*<LOD*			*<LOD*	
562 14 DAF Podwall	**33.32**	±	**1.82**	12.25	±	2.31	3.37	±	0.68	17.70	±	3.46	*<LOD*			*<LOD*	
562 16 DAF Podwall	**35.29**	±	**6.25**	9.78	±	2.14	4.12	±	0.33	21.39	±	4.03	*<LOD*			*<LOD*	
562 20 DAF Podwall	**29.37**	±	**4.03**	22.10	±	4.27	1.48	±	0.20	5.79	±	1.30	*<LOD*			*<LOD*	
Bolero 10 DAF Seedco	**137.76**	±	**14.76**	19.37	±	5.36	9.36	±	1.26	109.03	±	8.13	*<LOD*			*<LOD*	
Bolero 12 DAF Seedco	**94.56**	±	**23.20**	15.98	±	2.33	3.90	±	0.71	74.68	±	20.35	*<LOD*			*<LOD*	
Bolero 14 DAF Seedco	**767.86**	±	**28.58**	24.61	±	4.33	32.77	±	7.47	710.49	±	16.78	*<LOD*			*<LOD*	
Bolero 16 DAF Seedco	**74.38**	±	**10.35**	13.55	±	2.70	3.52	±	0.39	57.31	±	9.04	*<LOD*			*<LOD*	
Bolero 20 DAF Seedco	**83.50**	±	**16.95**	11.14	±	3.00	4.68	±	0.81	67.68	±	13.50	*<LOD*			*<LOD*	
562 10 DAF Seedcoat	**34.33**	±	**6.89**	9.06	±	1.58	3.22	±	0.80	22.06	±	4.87	*<LOD*			*<LOD*	
562 12 DAF Seedcoat	**331.84**	±	**92.39**	11.21	±	2.36	7.40	±	1.99	313.22	±	90.03	*<LOD*			*<LOD*	
562 14 DAF Seedcoat	**674.93**	±	**83.68**	28.64	±	5.50	21.27	±	1.05	625.02	±	80.44	*<LOD*			*<LOD*	
562 16 DAF Seedcoat	**1026.83**	±	**189.15**	19.07	±	4.67	39.79	±	10.43	967.96	±	196.50	*<LOD*			*<LOD*	
562 20 DAF Seedcoat	**821.76**	±	**193.17**	21.88	±	5.75	24.67	±	5.18	775.21	±	185.37	*<LOD*			*<LOD*	
Bolero 7 DAF Cotyledo	**81.23**	±	**11.85**	40.82	±	5.44	5.66	±	1.31	34.74	±	5.10	*<LOD*			*<LOD*	
Bolero 10 DAF Cotyled	**101.65**	±	**23.53**	16.19	±	3.23	11.03	±	1.81	74.43	±	18.75	*<LOD*			*<LOD*	
Bolero 12 DAF Cotyled	**18.64**			15.68	0.00		2.96				*<LOD*		*<LOD*			*<LOD*	
Bolero 14 DAF Cotyled	**35.87**	±	**7.95**	14.23	±	3.19	2.09	±	0.56	19.55	±	4.97	*<LOD*			*<LOD*	
Bolero 16 DAF Cotyled	**19.65**	±	**1.36**	10.70	±	0.97	0.93	±	0.16	8.02	±	1.14	*<LOD*			*<LOD*	
Bolero 20 DAF Cotyled	**40.61**	±	**0.97**	34.75	±	1.15	1.56	±	0.11	4.30	±	0.07	*<LOD*			*<LOD*	
562 7 DAF Cotyledon	**70.57**	±	**9.38**	18.51	±	1.94	4.20	±	0.30	47.86	±	7.14	*<LOD*			*<LOD*	
562 12 DAF Cotyledon	**34.49**			18.95			2.15			13.39			*<LOD*			*<LOD*	
562 14 DAF Cotyledon	**27.54**	±	**5.32**	7.08	±	1.84	0.74	±	0.08	19.72	±	4.90	*<LOD*			*<LOD*	
562 16 DAF Cotyledon	**37.64**	±	**2.24**	17.47	±	3.68	1.19	±	0.31	18.97	±	2.00	*<LOD*			*<LOD*	
562 20 DAF Cotyledon	**13.24**	±	**3.17**	11.03	±	3.14	0.41	±	0.08	1.79	±	0.12	*<LOD*			*<LOD*	