Running title: Iron signalling genes in legumes

1 Title: Genetic basis of historical pea mutants that hyper-accumulate iron

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8 Abstract

9 The Pisum sativum (pea) mutants degenerate leaves (dgl) and bronze (brz) accumulate large amounts 10 of iron in leaves. First described several decades ago, the two mutants have provided important insights into iron homeostasis in plants but the underlying mutations have remained unknown. Using 11 12 exome sequencing we identified an in-frame deletion associated with dql in a BRUTUS homologue. 13 The deletion is absent from wild type and the original parent line. BRUTUS belongs to a small family 14 of E3 ubiquitin ligases acting as negative regulators of iron uptake in plants. The brz mutation was 15 previously mapped to chromosome 4, and superimposing this region to the pea genome sequence 16 uncovered a mutation in OPT3, encoding an oligopeptide transporter with a plant-specific role in metal 17 transport. The causal nature of the mutations was confirmed by additional genetic analyses. Identification of the mutated genes rationalises many of the previously described phenotypes and 18 19 provides new insights into shoot-to-root signalling of iron deficiency. Furthermore, the non-lethal 20 mutations in these essential genes suggest new strategies for biofortification of crops with iron. 21 Keywords: iron / hemerythrin / RNA-seq / Pisum sativum / Medicago truncatula

Significance statement: Two iron-accumulating pea mutants first described more than 30 years ago have greatly contributed to our understanding of iron homeostasis in plants, but the mutations were never identified. Here we show that the phenotypes are caused by mutations in the *BRUTUS* and *OPT3* genes and how this leads to specific defects in iron signalling and leaf development.

Running title: Iron signalling genes in legumes

26 Introduction

27 Prior to the rise of Arabidopsis as a plant model organism, genetic studies in pea contributed greatly 28 to our understanding of the mechanisms of inheritance, and also helped identify several genetic loci 29 for morphological and nutritional traits in plants (Ellis et al, 2011). As part of these studies, two iron-30 accumulating pea mutants were independently isolated: the dgl mutant, from an X-ray mutagenized 31 population (Gottschalk, 1987), and the brz mutant, also named E107, generated using ethyl 32 methanesulfonate (EMS) (Kneen et al, 1990). In addition to a root nodulation phenotype, the leaves 33 of both mutants develop striking bronze spots and senesce prematurely (Fig. 1A, bottom panel). The 34 spots are necrotic tissue caused by toxic levels of iron, which accumulates 10 to 100-fold in older 35 leaves (Welch & LaRue, 1990; Fig. 1B, C). Despite the similar phenotypes, genetic analysis showed that 36 the brz and dql loci segregate independently (Kneen et al, 1990). The brz mutation was mapped to a large segment of chromosome 4, but was not further fine-mapped or cloned. For dgl, difficulties with 37 38 distinguishing heterozygotes within the F2 population due to variability in iron concentration thwarted 39 efforts to obtain any idea of its genome location (Kneen et al, 1990).



- 40 **Figure 1**. The *dgl* and *brz* mutants in pea (*Pisum sativum* L.) hyper-accumulate iron in leaves.
- 41 A. Top panel: Three-week old plants of the pea variety Sparkle (Sp) and the mutants *dgl* and *brz* in the same
- 42 genetic background. Bottom panel: Lower leaflet of 5-week-old plants, showing yellowing and brown spots.
- 43 Scale bar = 1 cm.
- 44 B. Iron staining (blue) of leaf pieces from 5-week-old plants. Scale bar = 2 mm.
- 45 C. Iron concentrations in leaflets of the same leaf, measured two weeks apart using a colorimetric assay. Dots
- 46 represent measurements of individual plants.

Running title: Iron signalling genes in legumes

Despite not knowing the mutated genes, seminal physiological studies of the *dgl* and *brz* mutants demonstrated the existence of a Fe³⁺ reductase activity induced by iron deficiency (Welch & LaRue, 1990; Grusak *et al*, 1990) well before the corresponding gene was cloned from Arabidopsis (Robinson *et al*, 1999). In addition, grafting wild-type shoots onto mutant roots and vice versa revealed the existence of a shoot-to-root signal for iron deficiency (Welch & LaRue, 1990; Grusak & Pezeshgi, 1996; García *et al*, 2013), the molecular nature of which is still unknown.

53 Because iron is toxic and controlled by tight homeostasis mechanisms, there is limited genetic 54 variation for the iron concentration in crops and mutant screens rarely turn up iron-accumulating 55 mutants (Connorton & Balk, 2019; Lahner *et al*, 2003). Therefore, identifying the *dgl* and *brz* mutations 56 could be important both for our general understanding of iron homeostasis and to help design 57 strategies for biofortifying crops.

Finding genetic loci is greatly facilitated by a genome sequence, but owing to its large size (~4.45 Gb), a draft of the pea genome was not published until recently (Kreplak *et al*, 2019). This confirmed the close relationship and extensive co-localization of genetic loci (synteny) with the *Medicago truncatula* genome. Making use of this new resource, we show that *dgl* is caused by a short deletion in *BRUTUS*, a putative iron sensor and negative regulator of iron uptake, and that *brz* is associated with mutations in the iron transporter *OPT3* in both pea and Medicago.

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65 Results and Discussion

66 *dgl* is associated with a small in-frame deletion in *BRUTUS*

67 To investigate the transcriptional basis of iron accumulation in the *dql* pea mutant and, if possible, to identify the causative mutation, we carried out RNA sequencing on leaves of *dgl* and the wild-type 68 cultivar Sparkle, into which the original dql mutation was introgressed by at least five backcrosses 69 70 (Marentes & Grusak, 1998). We found 86 differentially expressed genes which were highly enriched for genes involved in iron homeostasis (Suppl. Table 1 and 2). These included four different ferritin 71 72 genes with massively induced expression, in agreement with previous reports of increased ferritin 73 protein in *dal* leaves and seeds by electron microscopy, Western blot analysis and iron-stained native 74 gels (Becker et al, 1998; Marentes & Grusak, 1998). Also upregulated were vacuolar iron transporters 75 and a gene involved in zinc detoxification (PCR2), whereas various sugar metabolism genes are 76 downregulated (Suppl. Table 2).

Alignment of the RNA reads to the pea genome sequence (Kreplak *et al*, 2019) followed by
identification of SNPs and Indels in *dgl* relative to Sparkle confirmed that the lines were near-isogenic

Running title: Iron signalling genes in legumes

79 (Suppl. Fig. 1). Because the mutant was generated by X-rays, we focussed on the Indels as candidate 80 genetic polymorphisms (Table 1). Molecular marker analysis showed that 3 Indels located on 81 chromosome 6 were not linked to the mutant phenotype (data not shown). However, a deletion 82 located on chromosome 1 co-segregated invariably with the high-iron phenotype in the F_2 population 83 (n = 11 mutants out of 44 plants, Suppl. Fig. 2) and is not present in the original Dippes Gelbe Viktoria 84 pea variety used for mutagenesis (Fig. 2A). The 15-bp deletion is located in exon 2 of Psat1q036240, 85 corresponding to an in-frame deletion of five amino acids (Fig. 2B). A blastp search against the Arabidopsis proteome revealed that Psat1g036240 shares 66.4% identity with the BRUTUS (BTS) 86 87 protein from Arabidopsis, an E3 ubiquitin ligase that acts as a negative regulator of iron uptake (Selote et al, 2015; Rodríguez-Celma et al, 2019). The five deleted amino acids, QTSLS, are located in the first 88 89 hemerythrin motif (Fig. 2B) and are semi-conserved in BTS sequences across the green lineage. Loss 90 of these residues is predicted to displace two of the seven amino acid ligands that coordinate a diiron centre, thus potentially weakening iron binding and affecting the proposed oxygen and/or iron sensing 91

92 function of this domain (Fig. 2C; Suppl. Fig. 3).

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Chromosome / Linkage Group	Indel size	Gene	Outcome of mutation	Arabidopsis BLAST hits	Co-segre gates?
Chr1 / LG6	15 bp	Psat1g036240	In-frame deletion of TSLSQ	BRUTUS	Yes
Chr6 / LG2	24 bp	Psat6g181240	In-frame deletion of SMQQPLPS	Decapping 5 (DCP5)	No
Chr6 / LG2	15 bp	Psat6g182800	In-frame deletion of QTLPL	Mediator associated protein 1-like	No
Chr6 / LG2	21 bp	Psat6g183600	Insertion causes premature stop, deleting final VLVQ	No hits	No

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95 **Table 1**. Indels in *dgl* identified by exome mapping.

96 Four indels were identified as candidate X-ray mutation for *dgl* by comparing RNA-seq data from *dgl* and wild-

97 type variety Sparkle mapped to the pea genome (variety Cameor). Only the deletion on chromosome 1 co-

98 segregated with the *dgl* phenotype (see Suppl. Fig. 2).

Running title: Iron signalling genes in legumes



Figure 2. *dgl* is associated with a small in-frame deletion in *BRUTUS*.

A. PCR analysis with primers spanning the 15-bp deletion associated with *dgl* show that the deletion is absent
 from the near-isogenic wild-type Sparkle (Sp) variety and also from the Dippes Gelbe Viktoria (DGV) variety that
 was originally used for mutagenesis (Gottschalk, 1987).

B. The *dgl* mutant has a mutation in exon 2 of the gene *Psat1g036240*, encoding a BRUTUS homolog. The 15-bp

in-frame deletion removes five amino acids (QTSLS) of the first hemerythrin domain, close to two iron-binding
 residues (H and E) in a conserved HxxxE motif (in red).

C. Protein model of the Hr1 domain, with ligands binding the di-iron centre in magenta. The deleted 5 amino
 acids in the *dgl* mutant are rendered in white and marked with a white arrow. The deletion is predicted to
 displace two of the seven iron-binding ligands, see Suppl. Fig. 3.

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111 brz is associated with a point mutation in OPT3

112 The brz mutation was previously mapped to the tip of chromosome 4 between the phenotypic markers lat (latum, Latin for wide leaves) and was (waxy stipules) (Kneen et al, 1990; Ellis and Poyser, 2002; 113 114 Fig. 3A). These two loci have not been identified, but closely linked genes that have been cloned suggested that brz lies between Psat4g001240 and Psat4g005920, an interval of more than 450 genes. 115 116 Near the middle of this interval are the likely gene candidates *Psat4q003000* and *Psat4q003080*, paralogous genes encoding the oligopeptide transporter OPT3. This member of the large OPT 117 118 transporter family is unique to vascular plants and forms a separate phylogenetic clade with a single 119 origin. Mutant studies in Arabidopsis showed that OPT3 has an essential function in transporting iron 120 from the xylem to the phloem (Zhai et al, 2014; Mendoza-Cózatl et al, 2014) and also mediates copper 121 transport (Chia et al, 2023). Publicly available transcriptome data of pea suggests that Psat4q003000

Running title: Iron signalling genes in legumes

does not have meaningful expression levels whereas *Psat4g003080* is expressed in all plant organs, particularly in young leaves and stems (**Suppl. Fig. 4B**). Sequencing of the coding sequence identified a C > T mutation, consistent with the effect of EMS as a mutagen, that co-segregated with the ironaccumulating phenotype (**Suppl. Fig. 5**). The missense mutation changes a conserved leucine residue into phenylalanine (Leu466Phe, **Fig. 3B**). Protein modelling suggests that Leu466 is part of an alpha helix oriented inwards (**Fig. 3C**) and substitution to a larger, more hydrophobic side group may interfere with the transport mechanism.



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130 **Figure 3.** *brz* is associated with a point mutation in OPT3.

A. The *brz* mutation was previously mapped to the tip of chromosome 4, between the phenotypic loci *lat* and
 was (Kneen et al., 1990; Ellis & Poyser, 2002). Neigbouring loci that have been cloned were used to obtain a
 physical interval between genes *Psat4q001240* and *Psat4q005920*.

134 B. The *brz* mutant contains a C to T mutation in exon 4 of the gene *Psat4g003080*, encoding the oligopeptide

transporter OPT3, resulting in substitution of the highly conserved leucine 466 to phenylalanine.

136 C. Protein model of Arabidopsis OPT3, centred on Leu461 (equivalent to Leu466 in pea OPT3, yellow arrow).

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138 Further genetic evidence for the mutations of *dgl* and *brz*

139 To confirm that the identified mutations are causally linked to the *dgl* and *brz* phenotypes, we used

140 different approaches. First, genetic complementation in pea was attempted by transiently expressing

141 the wild-type pea *BTS1* cDNA in *dgl* seedlings, using *Agrobacterium*-mediated leaf infiltration. This did

142 not decrease or prevent iron accumulation in the infiltrated leaves, either because expression was too

143 low (GFP levels expressed using a separate construct were also low) or because iron accumulation in

this mutant is controlled systemically and cannot be suppressed locally. Stable transformation of pea

Running title: Iron signalling genes in legumes

has been reported but is technically challenging. Therefore, as a second approach, we applied TILLING 145

146 (Targeting Induced Local Lesions IN Genomes) to the syntenic Medicago genes. An EMS-mutagenized

147 population was screened for sequence polymorphisms in a 1.5 - 2 kb DNA region overlapping with or

close to, respectively, the position of the *brz* and *dql* mutations. 148

For OPT3, we isolated four non-synomymous mutations altering conserved amino acid residues. Of 149 those, Medicago plants with Pro529Leu or Pro618Leu presented with bronze spots on the leaves and 150 151 intense iron staining (Fig. 4A). Pro618Leu homozygous seedlings segregated at a low frequency and 152 had a severe growth phenotype, confirming the critical function of OPT3 in Medicago. For BTS1, an 153 unusually small number of mutations was found in the selected region (exons 7 and 8, corresponding 154 to the Hr 3 domain, Fig. 2C). Of the five non-synomymous mutations, only two affected conserved 155 amino acids. Asp912Asn had no discernable phenotype, whereas Asp730Asn homozygous plants could not be found among the offspring of a heterozygous plant. This suggests that BTS1 is an essential gene 156 157 in Medicago, similar to BTS being essential for embryo development in Arabidopsis (Selote et al, 2015), 158 and prevented further studies in young seedlings or mature plants.



B Wild type (Col-0)



+ Ps BTS1^{dgl}

Figure 4. Further genetic evidence for the mutated genes in dgl and brz.

A. Mutations in Medicago truncatula OPT3, which is syntenic with pea OPT3/Psat4g003080, phenocopy the pea brz mutant, including necrotic leaf spots and iron accumulation. Wild type (A17) and mutants in *OPT3* (*Medtr6g083900*), c.C1586>T (p. P529L) and c.C1853>T (p. P618L) grown on soil (top); close up of the leaves (middle); and leaf sectors stained for iron (insets). The dark purple spots in the middle of wild-type leaves are anthocyanin. Scale bars in the top panel are 1 cm.

B. The wild-type pea (Ps) BTS1 coding sequence genetically complements the iron accumulation phenotype of the Arabidopsis bts-3 mutant, but the pea BTS1-dql sequence does not. Detail of rosette leaves stained for iron, imaged by light microscopy. Scale bars are 0.2 mm. Genotyping data of the plants can be found in Suppl. Fig. 6B.

Running title: Iron signalling genes in legumes

159 Because no other exome sequence polymorphisms were found in close range of the 15-bp deletion 160 associated with dgl, it is rather unlikely that a different, yet genetically linked, mutation causes the 161 iron-accumulating phenotype. Nevertheless, as a third approach, we tested for complementation of 162 the Arabidopsis *bts-3* mutant (Hindt *et al*, 2017) with the coding sequences of wild-type pea *BTS1* or the *dal*-associated mutant gene. As a control, plants were transformed with Arabidopsis BTS. A 163 164 previous study showed that *bts-3* mutant plants, which carry a point mutation in the E3 ligase domain, 165 accumulated significant amounts of iron and had a severe growth defect (Hindt et al, 2017). We found 166 that in the T2 generation growth was variable, perhaps because of epigenetic effects. However, iron 167 accumulation visualized by Perls' staining, which was most prominent in the veins, was clearly 168 suppressed in *bts-3* plants transformed with *AtBTS* or pea *BTS1*, but not with the *dql*-associated variant 169 of BTS1 (Fig. 4B; Suppl. Fig. 6). These results demonstrate that pea BTS1 is a functional orthologue of 170 Arabidopsis BTS and that the 15-bp deletion is deleterious for BTS function.

Phenotypic differences between *dgl* and *brz* are associated with the specific functions of BTS and OPT3, respectively, in iron homeostasis

The pea *dgl* and *brz* mutants have an unusual history in that detailed reports of their phenotypes have 173 174 been published since 1990, which can only now be matched to the affected genes. While both mutants accumulate large amounts of iron in leaves, the pattern of accumulation differs in subtle ways. In the 175 176 brz / opt3 mutant, iron staining is restricted to the narrow ends of leaf veins, consistent with high 177 expression of the Arabidopsis OPT3 gene in the minor veins (Zhai et al, 2014) (Suppl. Fig. 7). By 178 contrast, the *dql* mutant accumulates iron in both minor and major veins, matching the promoter 179 activity of Arabidopsis BTS, which can reasonably be extrapolated to the pea BTS1 gene. dql mutants 180 also accumulate iron in the seeds, whereas brz seeds have less iron compared to wild type (Grusak, 181 1994; Marentes & Grusak, 1998). Seeds derive their iron stores from the phloem (Fig. 5), and iron 182 loading into the phloem is strongly impaired in opt3 mutants. Moreover, transcript levels of OPT3 are 183 very low in Arabidopsis seeds, whereas BTS is highly expressed in the embryo cotyledons (Selote et al, 184 2015; Zhai et al, 2014). From what we know of the function of BTS and its main ubiquitination target 185 ILR3 (Selote et al, 2015; Akmakjian et al, 2021), BTS suppresses the activity of the transcriptional cascade for iron uptake and, via ILR-PYE interaction, regulates iron remobilization (Fig. 5). dgl seeds 186 187 accumulate up to 4 times more iron, which is less than in leaves, probably reflecting a balance between 188 limited iron supply through the phloem (with iron not being remobilized from leaf veins) and upregulated iron uptake in the embryo. 189

Grafting experiments demonstrated the existence of a shoot-to-root signal leading to constitutive iron
uptake in the roots of *dgl* and *brz* mutants (Welch & LaRue, 1990; Grusak & Pezeshgi, 1996). The signal

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Running title: Iron signalling genes in legumes

192 is thought to be generated by iron deficiency in the phloem, but its molecular nature remains to be 193 identified. Interestingly, brz shoots also suppressed the number of symbiotic root nodules (Huynh & 194 Guinel, 2020) and it is possible that the same signalling molecule leads to both physiological outputs. 195 The role of neither BTS or OPT3 in iron homeostasis during nodule development has been properly 196 investigated (Day & Smith, 2021). opt3 mutants will be invaluable to shed light on the question of 197 whether iron is delivered to nodules via the xylem or phloem, or both. bts mutants could help lift the 198 lid on the regulation of iron homeostasis in nodules, including downstream transcription factors. Increasing the mineral micronutrient of crops, or biofortification, is an important area of research to

- 200 combat hidden hunger and to provide nutritious plant-based diets in the face of climate change. The 201 identified mutations indicate that either BTS or OPT3 function could be modified to increase the iron
- 202 content of vegetative tissues. Genetic variants of BTS could also enhance iron in seeds. However, there
- 203 is a fine balance between increasing iron and toxicity symptoms, and null mutants of BTS and OPT3
- 204 are embryo-lethal (Selote et al, 2015; Zhai et al, 2014; Mendoza-Cózatl et al, 2014). Interestingly, the
- 205 pea dal mutant has relatively minor growth defects, indicating that it is worth screening for weaker
- 206 alleles, especially in the Hr1 domain. In summary, knowing the causal mutations in the historic dal and
- 207 brz mutants will help to further unravel the functional roles of these important iron homeostasis 208 genes.



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Running title: Iron signalling genes in legumes

211 Reduced function of the E3 ubiquitin ligase BRUTUS (BTS) or the oligopeptide transporter OPT3 in the dql and 212 brz mutants, respectively, leads to constitutive activity of the iron uptake pathway in roots (Welch & LaRue, 213 Grusak et al, 1990). The pea BTS1 gene is expressed predominantly in shoots 1990; 214 (https://urgi.versailles.inra.fr/), in agreement with Arabidopsis BTS promoter activity and high transcript levels 215 in leaves, root stele and embryo cotyledons (Selote et al, 2016; Hindt et al, 2017). Arabidopsis BTS interacts with 216 the transcription factor ILR3 to target it for degradation (Selote et al, 2016), although evidence of ubiquitination 217 is currently lacking. Decreased activity of BTS leads to increased levels (and transcriptional activity) of ILR3, which 218 indirectly leads to enhanced transcription of iron uptake genes. Iron accumulates in minor and major veins and 219 also in seeds. AtOPT3 is expressed in the phloem (Zhai et al, 2014; Mendoza-Cózatl et al, 2014) and mediates 220 transport of iron and copper from the xylem into the phloem. Impairment of OPT3 function leads to 221 accumulation of iron in the xylem, in particular minor veins, and to decreased iron levels in the phloem and in 222 seeds. Iron deficiency in the phloem triggers a signal, the nature of which is still unknown, that induces the 223 expression of iron uptake genes in the roots and enhanced activity of the ferric reductase FRO2.

Running title: Iron signalling genes in legumes

224 Materials and Methods

225 Plant material and growth

226 Seeds of dgl (JI3085) and the wild type 'Sparkle' (JI0427) were obtained from the John Innes Centre Germplasm Resource Unit (GRU), which were donated to the collection by Michael Grusak, then at 227 Asgrow Seeds, Twin Falls, USA. Seeds of the EMS mutant E107, also named brz (JI2616) were also 228 229 obtained from GRU, donated by Thomas LaRue, Cornell University, Ithaca, USA. The germination rate 230 of brz seeds declines rapidly over time, and the mutant should be propagated every 3 - 4 years. The dal mutant was originally generated in the pea variety Dippes Gelbe Viktoria (DGV, accession JI2413 231 232 in the GRU collection). Marentes & Grusak (1998) backcrossed dgl at least five times with the Sparkle variety, selected at the F_3 stage for the high-iron phenotype. For segregation analysis, dql and brz were 233 234 crossed with line JI804 because of its contrasting phenotypic traits and suitable genetic markers. Plants 235 were germinated on peat-based compost (Levington F2) and grown in a greenhouse with additional 236 lighting in the winter and watering as required.

237 Leaf iron staining and quantification

- Leaf samples were stained for iron using Perls' reagent as previously described (Meguro *et al*, 2007). Samples were mounted in 50% (v/v) glycerol and imaged on a Axio Zoom.V16 stereo microscope with an Axiocam 512 color camera (Zeiss). For measuring iron concentrations, dried leaf samples were digested in 0.25 ml nitric acid (69% w/v) and 0.25 ml hydrogen peroxide (30% w/v) at 90°C. After neutralizing with 1 ml ammonium acetate (15% w/v), samples were reduced with 0.1 ml ascorbic acid (4% w/v). Fe²⁺ was quantified using the colorimetric iron chelator ferene (3-(2-pyridyl)-5,6-bis-[2-(5furyl-sulfonic acid)]-1,2,4-triazine, 0.1% w/v) and absorbance was measurement at 593 nm.
- 245 RNA extraction and Illumina sequencing

Leaf tissue was sampled from three plants of each genotype (Sparkle and *dgl*) and snap-frozen in liquid N₂. The frozen tissue was ground to a fine powder before RNA extraction using TRIzol[®] Reagent (ThermoFisher) and DNase treatment with TURBO DNase (ThermoFisher). The quality and quantity of RNA was verified with the Agilent Bioanalyzer RNA 6000 Nano assay before cDNA library preparation (250-300 bp insert) and Illumina Sequencing (PE 150, Novogene).

251 Differential expression analysis

252 Illumina reads were pseudo-aligned against the *P. sativum* reference transcriptome (Kreplak *et al*, 253 2019) using Kallisto (Bray *et al*, 2016). Gene expression levels were determined using the R package 254 Sleuth (Pimentel *et al*, 2017) using the Wald test, where we considered genes to be differentially 255 expressed between genotypes with q < 0.05. Enrichment of GO terms was calculated using the R 256 package goseq (Young *et al*, 2010).

Running title: Iron signalling genes in legumes

257 Identification of deletions

Illumina reads were aligned against the *P. sativum* reference genome (Kreplak *et al*, 2019) using the
software *BWA-mem* (Li, 2013). The software transIndel (Yang R & Van Etten JL, 2018) was then used

- to identify the location of deletions and insertions using the default parameters except for DP = 1 (to
- 261 capture all possible deletions, irrespective of gene expression level).
- 262 PCR analysis to confirm allelic variation

263 DNA was extracted from individual F₂ plants in 200 mM Tris-HCl pH 7.5, 250 mM NaCl, 25 mM EDTA and 0.5% (w/v) sodium dodecyl sulfate and precipitated with 50% (v/v) isopropanol. For dql, PCR 264 265 primers were designed to amplify across the region of *Psat1q036240* which contains the identified five codon deletion: GCGTGAAGAATGTAGCACAG and ACCTGCAATATTCAACCAGCA, see Suppl. Table 266 267 3. Amplified fragments were run on a 2% (w/v) agarose gel for 2 h at 70 V, allowing separation of the wild-type Sparkle band (334 bp) and the dgl band (319 bp). For brz, PCR primers were designed to 268 269 span part of the *Psat4q003080* coding sequence: GACATATTGAGACAGAGCAGG and 270 ATACCGAATCATGAACTGTGC, see Suppl. Table 3. The PCR product was purified and sequenced.

271 Protein homology modelling

The first hemerythrin domain of pea BTS1 / Psat1g036240.1 (amino acids 55 – 184), the *dgl* variant of this domain, the full length OPT3 protein and the L466F variant were modelled using AlphaFold2 and visualised using PyMol version 2.5.2.

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276 Genetic complementation of Arabidopsis bts-3

277 Heterozygous *bts-3* plants (Hindt *et al*, 2017) were transformed with plasmid plCSL869550OD (SynBio) carrying either the Arabidopsis BRUTUS coding sequence (BTS, AT3G18290); the pea BRUTUS1 coding 278 sequence (Psat1q036240); or the pea dql variant of BRUTUS1 (lacking nucleotides 487-510). The 279 280 coding sequences were placed downstream of the Arabidopsis BRUTUS promoter, nucleotides -1904 281 to -1, and upstream of the ocs terminator, using Golden Gate assembly. All constructs were verified by sequencing. T1 plants homozygous for *bts-3* were selected by PCR (see Suppl. Table 3 for primers) 282 283 followed by restriction with PfIMI, the recognition site of which is deleted in the *dql* allele. Of these 284 lines, T2 plants carrying the transgene were scored for growth and iron accumulation.

285 Medicago truncatula TILLING

A M2 population of EMS-mutagenized *Medicago truncatula* was screened for genetic polymorphisms in *BTS1/Psat1g036240* and *OPT3/Psat4g003080*. For *BTS1*, primers MtBTS1-F1 and -R spanned exons 7 - 10 to maximize the ratio of exon:intron sequence; For *OPT3*, primers MtOPT3-F1 and -R3 spanned exons 4 - 6, surrounding the *brz* mutation. See Suppl. Table 3 for primer sequences. For the selected

Running title: Iron signalling genes in legumes

- lines (Suppl. Table 4), seedlings were grown up and inspected for phenotypes and iron accumulation
- 291 using Perls' staining.
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Running title: Iron signalling genes in legumes

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Running title: Iron signalling genes in legumes

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Supplemental Figure 1. Exome mapping to identify the *dgl* mutation.

Circular representation of the pea (*Pisum sativum* L.) genome to which the RNA-seq data from *dgl* and Sparkle (wild type) are mapped. Blue lines represent sequence polymorphisms and yellow indicates sequence identity between *dgl* and Sparkle.



Supplemental Figure 2. Co-segregation analysis of the *dgl* mutation and iron accumulation in pea, *Pisum sativum* L.

A. The *dgl* mutant was crossed with a pea variety acting as wild-type for the locus (JI804), to obtain an F2 population of 44 plants.

B. Discs (3 mm diameter) of the second leaf were stained for iron and scored for the iron-accumulating phenotype.

C. PCR analysis of selected F_2 plants, *dgl* and the wild-type control Sparkle (Sp), to detect the 15-bp deletion in *Psat1g036240* as well as the wild-type allele.



Supplemental Figure 3. Models to show the position of amino acid ligands of the diiron centre in the hemerythrin 1 domain, in wild-type pea BRUTUS (left) and as a consequence of the 5-aa deletion in *dgl* (right). The diiron centre is predicted to have 7 ligands (plus water or oxygen) following a pattern that is conserved in all hemerythrins (H...HxxxE...H...HxxxE), consisting of histidine (His, H), glutamate (Glu, E) plus one glutamine (Gln). Because of the 5 amino acid deletion in the *dgl* mutant, the nearby His169 and Glu173 are predicted to be displaced and pointing away from the active site.



Expression levels (TPM)

	Root	Nodule	Stem	Lower leaf	Upper leaf	Flowers	Pods	Young seeds (12 dap)
Psat4g00 3000	0	0	0.01	0	0.01	0	0.01	0
Psat4g00 3080	3.39	9.08	19.44	16.63	28.58	12.35	3.49	12.29
				e		(5)		

https://urgi.versailles.inra.fr/download/pea/Pea_PSCAM_transcriptome/

Supplemental Figure 4. The pea homolog OPT3 is a candidate gene for BRZ.

A. The *brz* mutation was previously mapped to the tip of chromosome 4, between the genetic markers *lat* and *was* (Kneen et al., 1990; Ellis & Poyser, 2002).

B. Detail of chromosome 4 showing the two neighboring *OPT3* paralogs, *Psat1g003000* and *Psat1g003080*. Expression data in transcripts per million (TPM) from https://urgi.versailles.inra.fr/ indicate that only one of the two paralogs, *Psat1g003080*, is expressed.

А								В		
0	12	0	3	(1)	3	0	0			<i>PsOPT3</i> , position c.1396
	-		3				DIE	Γ	1-2	С
		-	-				12	Γ	1-3	C/T
		100						Γ	1-4	C/T
				De la		3	an (G		1-5	C/T
15		-			-		312		1-6	Т
-									1-7	С
0	0								1-8	Т
4.4	4 7	1 1 2	2.5	0.11	2.2	///	C n		1-12	Т
1-1	1-7	1-13	2-5	2-11	3-3	3-9	Sp		2-2	С
1-2	1-8		2-6	2-12	3-4	3-10	Sp		2-5	Т
1-3	1-9	2-1	2-7	2-13	3-5	3-11	brz		2-7	Т
						• • • •			2-9	C/T
1-4	1-10	2-2		2-14		3-12	brz		2-12	C/T
1-5	1-11	2-3	2-9		3-7	3-13	JI804		3-3	Т
1.0	4.40	0.4	0.40	2.0	2.0	0.44			Sp	С
1-6	1-12	2-4	2-10	3-2	-১	3-14			brz	Т

Supplemental Figure 5. Co-segregation analysis of the *brz* mutation and iron accumulation in pea, *Pisum sativum* L.

A. Iron-stained leaf discs (3 mm diameter) of F_2 plants from crosses between *brz* and JI804 used as wild type. The F_2 were from 3 different F1 plants. B. Allele variation for c.1396 in *Psat4g003080 / PsOPT3* in 14 F_2 plants, wild-type Sparkle (Sp) and *brz* serving as negative and positive controls, respectively.



Supplemental Figure 6. Genetic complementation of the Arabidopsis *bts-3* mutant with pea *BTS1*. **A**. Heterozygous Arabidopsis *bts-3* plants were transformed with T-DNAs containing cDNA sequences of Arabidopsis *BTS* (*AtBTS*), pea (*Ps*) *BTS1* and the *dgl*-associated variant of pea *BTS1* (*PsBTS1-dgl*). T1 plants homozygous for *bts-3* were selected, and T2 seedlings from these plants were grown up. Leaf discs (3 mm) of older leaves were stained for iron and imaged by light microscopy. Representative images of 2 independent lines are shown. Scale bar is 0.5 mm.

B. Genotyping of the plants pictured in Fig. 4, using PCR. The *bts-3* mutation removes a PfIMI restriction site, detected by PCR followed by PfIMI treatment. Sizes of the nucleotide bands are on the right. Primers are listed in Suppl. Table 3.



Supplemental Figure 7. Pattern of iron accumulation in the leaves of *dgl* and *brz* mutants. Leaves were stained using the Perls' method and imaged using a stereo microscope and bright-field illumination. Larger veins (arrows) accumulate iron in the *dgl* mutant but not in the *brz* mutant.

Supplemental Table 1. Gene Ontology terms of differentially expressed genes in leaves from the pea dgl mutant compared to the corresponding wild-type variety Sparkle. BP, biochemical pathway; MF, molecular function.

category	term	ontology	over_rep_padj
GO:0006826	iron ion transport	BP	3.58E-07
GO:0006879	cellular iron ion homeostasis	BP	4.95E-06
GO:0008199	ferric iron binding	MF	4.95E-06
GO:0055072	iron ion homeostasis	BP	4.95E-06
GO:0000041	transition metal ion transport	BP	1.28E-05
GO:0030003	cellular cation homeostasis	BP	2.25E-05
GO:0055082	cellular chemical homeostasis	BP	2.25E-05
GO:0006873	cellular ion homeostasis	BP	2.64E-05
GO:0050801	ion homeostasis	BP	8.73E-05
GO:0055080	cation homeostasis	BP	8.73E-05
GO:0048878	chemical homeostasis	BP	0.000207
GO:0030001	metal ion transport	BP	0.005288

Supplemental Table 2. Differentially expressed genes in leaves from the pea dgl mutant compared to the corresponding wild-type variety Sparkle.

Came opation Bit Arabboopsis BLASTy ht TPH (g) TPM (g) <thtpm (g)<="" th=""> <thtpm (g)<="" t<="" th=""><th></th><th></th><th></th><th></th><th></th><th>Change in TPM</th></thtpm></thtpm>						Change in TPM
Partig Trans. Trans. <thtrans.< th=""> <thtrans.< th=""> <thtrans.< td="" td<=""><td>Gene</td><td>a-value</td><td>Rest Arabidonsis BLASTy hit</td><td>TPM dal</td><td>TPM Sparkle</td><td>between dgl and</td></thtrans.<></thtrans.<></thtrans.<>	Gene	a-value	Rest Arabidonsis BLASTy hit	TPM dal	TPM Sparkle	between dgl and
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Pendig 19800 425-23 Nove 003 000 003 0003 0003 0003 0003 0003	Psat1g112240	3.30E-23	AT5G51560	7.86	0.27	7.59
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Bath 2017 201 4 a 15-01 FERSTIN(1) (ATSQU (SQU)) 343 72 77.43 355 72 Details 1260000 3.81 6-04 TLOOP 20 0.00 6.44 -6.46 Paul4 116720 S.27E-04 Strot This to multiple ARF proteins 0.00 0.53 -6.46 Paul2 127700 1.486-04 Strot This to multiple ARF proteins 17.53 0.00 0.59 Paul2 127500 1.37E-06 None 24.54 3.66 20.86 Paul2 127500 1.37E-06 None 24.54 3.66 20.86 Paul5 1005000 1.77E-06 IAAB (ATSC22870) 144.55 2.246 -13.81 Paul5 1005000 1.37E-06 IAAB (ATSC22870) 0.00 1.31 1.31 Paul5 1026000 1.37E-06 IAAB (ATSC22870) 0.00 1.31 1.31 Paul5 124200 2.54E-06 ATSC355800 9.02 1.55.1 4.58 Paul5 124200 2.54E-06 ATSC355800 9.02 1.51.3 4.51 Paul5 124200 0.00027 FELS (TTGC0720) 7.62 2.44 5.38 Paul5 124200 0.00027 FTGC0720 0.02 1.42	Psat2g035000	1.14E-07		1.79	0.01	1.78
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Panday 18720 5.27E-06 Short hits to multiple ARF proteins 0.00 3.30 3.33 Panday 12500 1.38E-06 None 2.4 5.4 8.6 2.0 8.6 Panday 15500 1.38E-06 None 2.4 5.4 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 8.6 2.0 2.0 1.6 2.6 2.6 2.6 1.6 2.6 2.6 1.6 2.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6 1.6 2.6<	Psat0s126g0080	3.81E-06	AT1G06720	0.00	6.46	-6.46
Talkagi (2000) 1.148 0.138 0.145 3.26 1.145 5.36 0.145 3.370 0.66.02 Partidg 104800 1.372-05 RP3 (AT3.004800) 0.00 0.00 1.13 -1.14 -1.44 -3.84 -3.84 -3.84 -3.85 -3.34 -3.84 -3.84 -3.84 -3.84 -3.84 -3.84 -3.84 -3.84 -3.84 -3.84	Psat4g186720	5.27E-06	Short hits to multiple ARF proteins	0.00	3.30	-3.30
Paradag 125800 171E-055Nort INIS to multiple ARP proleins 17.753 0.00 17.753 Paradag 1225800 1.77E-055N5005000 1.11 6.5.4 -5.43 Paradag 1225800 1.77E-055N505000 9.02 3.7.0 66.02 Paradag 125800 1.77E-055N20500 9.02 3.7.0 66.02 Paradag 125800 0.97E-053N20 9.00 1.13 -1.13 Paradag 125200 0.97E-053N20 6.0 1.13 -1.13 Paradag 125200 0.97E-053N20 0.0 1.13 -1.13 Paradag 125200 0.00023FBis (ATSG01720) 7.82 2.44 5.38 Paradag 1253000 0.00023FBis (ATSG01720) 2.7.3 51.63 -2.43 Paradag 125000 0.00023FAI-057020 2.7.3 51.63 -2.43 Paradag 145000 0.00023FAI-057020 2.7.3 51.63 -2.43 Paradag 145000 0.00023FAI-057070 3.8 1.8 -4.00 Paradag 145000 0.00023FAI-057070 3.8 1.8 -4.00 Paradag	Psat2g127760 Psat7g253920	1.48E-05 1.53E-05	A13G29180 None	0.00	0.59	-0.59
Paulsg12280 1.71E-05ATSG0500 1.11 6.54 5.43 Paulsg1205000 1.78E-05RP3162(AT4C09800) 99.72 33.70 66.02 Paulsg104800 1.87E-05RP3162(CAT4C09800) 90.72 33.70 66.02 Paulsg104800 6.97E-05RP316(CAT4C09800) 0.00 1.13 -1.13 Paulsg104800 6.97E-05RP316(CAT4C09800) 0.00 1.13 -1.13 Paulsg104800 0.00017FERRITNA (AT3G11000) 352.03 107.15 2.44 67 Paulsg104800 0.00023None 0.01 1.94 -1.92 Paulsg105000 0.00023None 0.01 1.94 -1.92 Paulsg1670800 0.00023None 9.21 1.44 5.3 Paulsg167000 0.00057AT1G7220 0.012 1.42 -1.40 Paulsg167000 0.00057AT1G7220 0.012 1.42 -1.40 Paulsg167020 0.00023None 9.27 3.34 9.27 Paulsg167020 0.00023None 9.27 3.44 2.75 1.44 4.53 Paulsg169020	Psat4g165600	1.71E-05	Short hits to multiple ARF proteins	17.53	0.00	17.53
Pastig26600 1.7/EC.05/AV& (NT26/25/0) 9.7/E. 33.70 66.02 Pastig106801 3.2/E.05/AVG (NT26/25/0) 9.7.2 33.70 66.02 Pastig105801 2.2/E.05/AVG (NT26/05/0) 9.7.2 33.70 66.02 Pastig105801 0.2/E.05/AVG (NT26/05/0) 9.6.0 6.7.4 18.13 4.41 Pastig142690 8.7/E.05/PKG (AT26/1933) 10.01 Pastig142690 0.00023FEL3 (AT26/1933) 7.42 Pastig203020 0.0007/AT16/720100 32.03 107.15 2244 Pastig150580 0.00023FEL3 (ATSC01720) 7.82 2.44 5.38 Pastig150580 0.00027/AT16/720 0.02 Pastig150580 0.00057/AT16/720 0.2.3 S.5.83 0.42 Pastig150580 0.00037/AT16/720 Pastig160580 0.00023/AT16/720 Pastig160580 0.00023/AT16/720 0.2.3 S.5.83 0.42 Pastig150580 0.00037/AT16/720 Pastig160580 0.00037/AT16/720 Pastig160580 0.00037/AT16/720 Pastig17000 0.00037/AT16/720 Pastig17000 0.00027/AT16/720 Pastig160580 0.00037/AT16/720 Pastig17000 0.00027/AT16/720 Pastig16070 0.00027/AT16/720 Pastig17000 0.00027/AT16/720 Pastig17000 0.00027/AT16/720 Pastig1720 0.00027/AT16/720 Pastig1720 0.00027/AT16/720 Pastig1720 0.00027/AT16/720 Pastig1720 0.00027/AT16/720 Pastig1717000 1.6.4 Pastig172 0.000226EER/TINI (AT5C01600) 147.83 5.13 142.70 Pastig1720 0.00025/AT16/143 14.0 Pastig14720 0.00025/AT16/4103 14.6 2.96 Pastig1774 0.00023/AT6/4403 14.6 2.96 Pastig1774 0.000 Pastig17470 0.00025/AT6/4403 14.6 2.96 Pastig1774 0.000 0.0033Nome 1.0 0 0.96 12.7 Pastig067920 0.0035FLA1(AT5G0170) 2.24 2.2.82 2.42 2.82 2.42 2.82 Pastig17640 0.0033Nome 1.0 0.0 96.4 3.8 0 9.6 3 0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0 0.0	Psat5g129280	1.71E-05	AT5G05600	1.11	6.54	-5.43
Participasci Partitopasci Participasci Participasci Participasci Particip	Psat5g295600	1.76E-05	IAA8 (AT2G22670)	14.55	32.96	-18.41
Paid6 j14320 5.54E-05.AEL/P32 (AT3G05680) 0.00 1.13 1.13 Paid1 j142800 6.9TE-06None 67.24 18.13 49.10 Paid1 j142800 0.00027FE3.(AT3G1150) 352.03 107.15 24.44 7.44 Paid1 j142800 0.00027FE3.(AT3G1150) 352.03 107.15 24.44 5.38 Paid1 j15200 0.00027FE3.(AT3G1120) 7.82 2.44 5.38 Paid1 j15200 0.00027Nne 0.01 1.94 1.132 Paid1 j15200 0.00057 AT116/7220 2.73 5.16.3 -24.30 Paid1 j15200 0.00057 None 9.55 0.34 9.27 Paid0 j15000 0.00126 AT3G27700 116 4.28 9.00 Paid0 j15000 0.00126 AT3G27700 147.83 5.13 142.73 Paid1 j15000 0.00226 FLAT (AT1G3170) 3.64 12.85 9.00 Paid2 j14000 0.00226 FLAT (AT1G3170) 1.46 2.96 1.53 Paid2 j24270 0.00226 FLAT (AT1G3170) 1.46 2.96 1.50	Psat5d068840	3.24E-05	AT3G56360	99.72	105.01	-95.99
Pastbg18820 6.91E-05None 67.24 18.13 49.10 Pastlg030260 8.77E-05PIRL6 (AT2G19330) 10.61 3.14 7.48 Pastlg030260 0.00017FERRINIX (AT3G11050) 352.03 107.15 24.44 Pastlg150500 0.00027 AT1G67020 0.02 1.42 -1.40 Pastlg150500 0.00057 AT1G67020 2.7.33 51.63 -24.30 Pastlg150500 0.00057 None 9.55 0.34 9.21 Pastlg127000 0.00057 None 9.55 0.34 9.21 Pastlg127000 0.00057 None 9.55 0.34 9.21 Pastlg127000 0.00057 None 16.79 4.23 4.60 Pastlg124000 0.000354 NG2046700 14.78 5.1 14.270 Pastlg141302 0.000254 NT3G14703 3.64 1.42 7.8 Pastlg1240400 0.000354 NG14 NT5G15000 14.47 8.51 14.270 Pastlg1216302 0.00035 None 1.40 6.71 4.973 Pastlg123170400 0.000350 None	Psat6g194320	5.54E-05	AtRLP33 (AT3G05660)	0.00	1.13	-1.13
Path 1942090 0.071 FERRTING (ATCS)19330) 1011 3.14 7.44 Pash 294303200 0.00022FR12, (ATSG)1720) 7.22 2.44 5.38 Pash 294303200 0.00027FR12, (ATSG)1720) 7.22 2.44 5.38 Pash 19150300 0.00027FR10 0.002 1.4 -1.40 Pash 29130200 0.00057FA1607200 2.733 5.163 -24.30 Pash 29140200 0.00057FA1607200 1.764 4.453 -26.80 Pash 29140200 0.00057FA16404760) 1.764 4.453 -26.80 Pash 29142000 0.000297FA1640430 1.764 4.453 -26.80 Pash 29142000 0.00129AT5C32760 16.79 2.73 41.66 Pash 29142700 0.00224FERTINI (AT1G31770) 3.84 12.25 -9.00 Pash 29142700 0.00226FERTINI (AT1G3170) 1.46 2.96 -1.50 Pash 29142720 0.00226FERTINI (AT1G3170) 1.24 2.73 4.66 Pash 2914272400 0.00238FER 1.455 -1.50 -1.50 -1.50 </td <td>Psat6g188320</td> <td>6.91E-05</td> <td>None</td> <td>67.24</td> <td>18.13</td> <td>49.10</td>	Psat6g188320	6.91E-05	None	67.24	18.13	49.10
sam Description Description Description Parist p162800 0.00023 None 0.01 1.94 1.92 Parist p162800 0.00057 ATT IGG7020 0.02 1.42 1.40 Parist p162800 0.00057 ATT IGG7020 27.33 51.63 -24.30 Parist p1628000 0.00057 AULLOC (AT2C46750) 1.6 7.93 -27.33 51.63 -24.30 Parist p127000 0.00102 WDL1 (AT3G0450) 17.64 44.53 -26.86 -27.33 51.31 -27.33 14.06 -28.33 -51.33 -27.33 14.06 -28.33 -27.33 14.06 -28.33 -27.33 14.06 -28.33 -27.33 14.06 -28.33 -27.33 14.06 -28.33 -27.33 14.06 -28.33 -27.33 14.06 -28.33 -27.33 14.06 -28.43 -27.33 14.06 -28.43 -23.33 -27.14 -27.33 14.06 -28.43 -23.23 -27.44 -28.43 -25.33 -27.21 -28.44 -28.44 -28.44	Psat1g142960 Psat2g030280	8.77E-05	PIRL6 (AT2G19330) EERRITIN2 (AT3G11050)	10.61	3.14	7.46
Peart 195200 0.00023 None 0.01 1.94 -1.92 Pearts 195300 0.00057 ATT GG720 20.2 1.42 1.40 Pearts 1950020 0.00057 NT GG720 27.3 5.1.63 -24.30 Pearts 195700 0.00057 None 9.55 0.34 9.21 Pearts 3850g0000 0.00057 None 17.64 44.53 -26.89 Pearts 127 10000 0.01128 AT5G23760 16.79 2.7.3 14.60 Pearts 121 40000 0.00128 AT5G23760 16.79 2.7.3 14.60 Pearts 113200 0.00228 HERTITH I (AT5G04600) 147.83 5.1.3 142.20 Pearts 113200 0.00228 HERTITH I (AT5G01600) 147.83 5.3.3 142.70 Pearts 113200 0.00228 HERTITH I (AT5G0170) 2.4 2.2.4 2.2.6 1.5.0 Pearts 199320 0.0033 None 13.70 0.96 1.2.74 Pearts 199320 0.0038 HEA11 (AT5G0170) 2.24 2.2.2 2.2.6 2.8.6 6.2.7 1.9.68 3.9.9.8 3.9.9.3 5.3.3 3.9.9	Psat7q249400	0.00023	FBL3 (AT5G01720)	7.82	2.44	5.38
Peatsg169360 0.00057AT1G67020 0.02 1.42 -1.40 Peatsg1690200 0.00057AT1G67020 27.33 55.16.3 -24.30 Peatsg1690200 0.00057AULGC6(A12C46760) 0.10 0.808 -7.88 Peatsg140200 0.00057AULGC6(A12C46760) 17.64 44.33 -26.89 Peatsg14720 0.0023AUTGC6(A1(A11G3770) 3.84 12.85 -3.00 Peatsg144000 0.00133ABCG14 (A11G3770) 3.84 12.85 -3.00 Peatsg14001 0.002250L74G1 (A11G3770) 3.84 12.85 -3.00 Peatsg14001 0.002250L74G1 (A11G3770) 4.66 2.96 -1.50 Peatsg14001 0.002250L74G1 (A10 -1.66 -1.60 -1.60 Peatsg1499320 0.00335None 17.40 .96 12.44 Peatsg1499320 0.0035251L741 (A15G0710) 2.24 2.28 2.26 2.85 5.67 2.16 3.53 Peatsg1919420 0.0063251L741 (A15G0740) -3.70 9.03 -5.33 5.46 4.46 2.955 3.840	Psat1g162800	0.00023	None	0.01	1.94	-1.92
Pathgl 100200 0.00037 None 2.73 3.0133 -24.30 Pathgl 27000 0.00037 None 9.35 0.34 9.21 Pathgl 34700 0.00037 None 9.35 0.34 9.21 Pathgl 34700 0.00128 AT 254760 17.764 44.53 -26.89 Pathgl 127 1g000 0.00128 AT 5522760 13.701 16.79 2.7.7 14.50 Pathgl 13200 0.002354 DECRIMIN (A15601600) 14.783 14.63 -53.83 Pathgl 13200 0.00226 AT 367 (H10117090) and UGE3 (AT 1663180) 40.80 9.463 -53.83 Pathgl 13200 0.00226 AT 367 (H10117090) and UGE3 (AT 1663180) 40.80 9.643 -54.93 Pathgl 13200 0.00238 None 17.40 67.13 -90.79 -90.63 -94.94 Pathgl 13200 0.00338 None 13.70 0.66 12.74 Pathgl 133.70 0.66 -22.42 -20.58 Pathgl 13300 0.00237 (Nane 13.70 0.66 -9.06 -9.68 -9.68 -9.68 Pathgl 13.710 -66 -7.98	Psat5g159360	0.00057	AT1G67020	0.02	1.42	-1.40
PartB3850g0080 0.00057C0ULO6 (AT2C46760) 0.10 8.08 7.98 PartBg14271g0080 0.00122WD1 (AT3C04830) 17.64 44.63 -26.88 PartBg14271g0080 0.00128ABCG14 (AT1C31770) 3.84 12.85 -9.00 PartBg124720 0.00228LGEE (IAT1C162770) 3.84 12.85 -9.00 PartBg124720 0.00228LGEE (IAT1C162780) and UGE3 (AT1G63180) 40.80 94.63 -53.83 PartBg124720 0.00228ATG14103 1.44 2.66 -15.55 PartBg124720 0.00228ATG14103 1.44 2.66 -15.55 PartBg129120 0.0023ATG14103 1.44 2.66 -15.55 PartBg129120 0.0033None 0.00 6.4 -9.64 PartBg1291200 0.0033None 0.00 6.4 -9.64 PartBg1291200 0.0033None 0.03 1.74 6.7.1 -2.74 PartBg1291200 0.00825CD1 (AT1G4040) 2.24 22.85 6.27 2.16 33.51 PartBg1291200 0.00825CD1 (AT1G406420) 4.55	Psat5g160280	0.00057	None	27.33	0.34	-24.30
PastBg144720 0.00102WDL1 (AT3G0480) 17.64 44.53 -26.89 PastBg142700 0.00192AT5G23700 13.64 12.85 -9.00 PastBg140800 0.00192AAECG14 (AT1G3770) 3.84 12.85 -9.00 PsatBg124720 0.00228FERRITINI (AT500100) 147.83 5.13 142.70 PsatBg13920 0.00228FERRITINI (AT500100) 147.83 5.13 142.70 PsatBg12020 0.00228FAT3G14940 3.06 39.81 -36.67 PsatBg12020 0.00250A14G14103 1.46 2.96 -1.50 PsatBg199320 0.0038SNone 13.70 0.96 12.74 PsatBg199320 0.0038SNone 3.70 0.93 10.36 -9.98 PsatBg199320 0.0038SNone 2.37 0.903 -5.33 PsatBg19320 0.0068SCD1 (AT16G60490) 0.38 10.36 -9.98 PsatBg199320 0.0068SCD1 (AT16G60490) 3.70 9.03 -5.33 -5.33 PsatBg1913120 0.0068SCD1 (AT16G60490) 3.70 9.03 -5.43 -9.45 </td <td>Psat0s3850g0080</td> <td>0.00057</td> <td>GULLO6 (AT2G46760)</td> <td>0.10</td> <td>8.08</td> <td>-7.98</td>	Psat0s3850g0080	0.00057	GULLO6 (AT2G46760)	0.10	8.08	-7.98
Patts 12/19080 0.00129A ISC23760 16.79 2.73 14.08 Patts 114000 0.0013A ABCC14 (AT1G31770) 3.84 12.85 4.900 Pasts 114720 0.00225 UEC1 (AT1G32780) and UEG3 (AT1G63180) 40.80 94.63 -53.83 Pasts 113920 0.00225 AT3G19430 10.62 (AT1G32780) 40.80 94.63 -53.83 Pasts 113920 0.00250 AT4G14103 14.6 2.96 -1.50 Past 2022880 0.00270 None 17.40 67.13 -4.973 Past 20220880 0.00270 None 0.00 9.64 -9.64 Past 2020800 0.00233 None 0.00 9.64 -9.64 Past 279000720 0.00385 FLA11 (ATSG03170) 2.24 2.28 -20.58 Past 279109720 0.00610 None 225.85 6.27 219.58 Past 279109720 0.00628 None 86.35 29.95 38.40 Past 29109720 0.00628 None 225.85 6.27 219.58 Past 29109720 0.00689 None 86.35 29.95 38.40	Psat6g144720	0.00102	WDL1 (AT3G04630)	17.64	44.53	-26.89
Passing 124720 0.00225ETERRITINI (ATSOUTGOU) 147.83 1.4.73 1.4.27 Passing 124720 0.00225EUGET (ATTGSOUTGOU) 147.83 9.4.3 5.3.83 Passing 13201 0.00225EUGET (ATTGSOUTGOU) 3.06 3.84 3.6.76 Passing 13201 0.00225EUGET (ATTGSOUTGOU) 3.06 3.84 3.6.76 Passing 13202 0.00225EUGET (ATTGSOUTGOU) 3.06 3.84 3.6.76 Passing 13202 0.00235INone 17.40 67.13 .4.9.73 Passing 107620 0.00333None 13.70 0.96 12.74 Passing 107620 0.006225EL14 (ATGSOUTGOU) 0.38 10.36 -9.98 Passing 107620 0.0061None 22.24 22.85 6.27 21.95 Passing 10720 0.0062825CD1 (ATIG49040) 3.70 9.03 -5.33 Passing 19380 0.00975Vaccular iron transporter homolog 2 (ATIG76800) 46.87 11.92 3.84 Passing 19380 0.00988PFP-ALPHAI (ATIG20550) 0.00 0.55 -0.55 Passing 19380 0.01130 None	Psat0s12/1g0080 Psat2g146080	0.00129	A15G23760 ABCG14 (AT1G31770)	16.79	2.73	14.06
PastTg1202 0.00235UGE1 (AT1G12780) and UGE3 (AT1G63180) 40.80 94.63 -53.83 Psatbg113202 0.0026A73G144103 3.06 39.81 -36.76 Psatbg214640 0.00250AT4G14103 1.46 2.96 -1.50 Psat2020880 0.00270None 1.74 67.713 -49.73 Psat2020802 0.00338None 13.70 0.96 12.74 Psat5p1905200 0.00320FLA12 (AT5G60490) 0.38 10.36 -988 Psat5p19057020 0.00620FLA12 (AT5G60490) 0.38 10.36 -988 Psat5p197640 0.00620FLA12 (AT5G60490) 68.35 2.93 63.84 Psat5p197640 0.00683None 68.35 2.93 63.46 Psat5p1197640 0.00683None 68.35 2.93 63.46 Psat5p1198200 0.00898ACCD1 (AT1G4900) 16.86 0.00 16.88 Psat5p1198200 0.00898ACCD1 (AT1G50520) 10.03 8.75 1.745 Psat5p12915100 0.01130MCT1 (AT4629330) 46.87 11.92 3.45	Psat3g124720	0.00226	FERRITIN1 (AT5G01600)	147.83	5.13	142.70
Peatbg113920 0.00245A13G19430 3.06 39.81 -36.76 Peatbg214640 0.00250A14G14103 1.46 2.96 -1.50 Peatbg214640 0.00250A14G14103 1.46 2.96 -1.50 Peatbg199320 0.00385None 0.00 9.64 -9.64 Peatbg199320 0.00385None 2.24 2.28.2 -20.58 Peatbg197640 0.00610None 2.25.85 6.27 219.58 Peatbg197640 0.00680None 2.25.85 6.27 219.58 Peatbg197640 0.00688None 2.65.67 1.21.6 3.3.51 Peatbg197640 0.00688None 2.95.6 3.8.40 1.68 0.00 16.89 Peatbg198280 0.00989PFP-ALPHA1 (A11620950) 0.00 0.55 -0.55 Peatbg198280 0.0170WRKY51 (A1566410) 7.38 2.12 5.26 Peatbg19216120 0.01313None 2.29 0.00 2.29 Peatbg192200 0.01685RPF (A11620950) 7.1.36 2.12 5.26 Peatbg19221440	Psat7g036120	0.00235	UGE1 (AT1G12780) and UGE3 (AT1G63180)	40.80	94.63	-53.83
Past2g124860 0.00270None 1.46 2.96 -1.30 Past2g12860 0.00270None 17.40 67.13 4.97.3 Past2g129300 0.00383None 13.70 0.96 4.274 Past2g12920 0.00385PLA11 (AT5G03170) 2.24 2.282 -2.058 Past3g19300 0.00520FLA12 (AT5G60490) 0.38 10.36 -9.98 Past3g197640 0.00682SCD1 (AT1G49040) 3.70 9.03 -5.33 Past3g120880 0.00898CCD1 (AT1G49040) 3.70 9.03 -5.33 Past3g13080 0.00975Vacuolar iron transporte homolog 2 (AT1G76800) 46.87 11.22 -4.65 Past3g19136120 0.00989CCD1 (AT3G64520) 16.88 0.00 16.88 Past3g12120 0.01130ERT (AT4629330) 46.87 11.122 3.49 Past3g129200 0.01133AMC1 (AT4629330) 46.87 11.122 3.49 Past3g129200 0.01130ERT (AT4629330) 46.87 11.32 2.87 Past3g129200 0.0133AMC1 (AT1602170) 11.30 2.87 17.45 <td>Psat4g113920</td> <td>0.00245</td> <td>AT3G19430</td> <td>3.06</td> <td>39.81</td> <td>-36.76</td>	Psat4g113920	0.00245	AT3G19430	3.06	39.81	-36.76
PastTg23404 0.003/s1None 0.00 0.64 9.64 PastTg1067920 0.00385Ren 13.70 0.96 12.74 PastTg1067920 0.00385FLA11 (AT5G03170) 2.24 22.82 -20.58 PastG1919704 0.00610Nene 22.58 6.27 21.95 PastG19177040 0.006610Nene 22.58 6.27 21.95 PastG1910780 0.006689Nene 68.35 29.95 38.40 PastG1910780 0.00698PPP-ALPHA1 (AT1520950) 16.98 0.00 16.58 PastG202400 0.01130DERT (AT426320) 46.87 11.92 34.95 PastG202400 0.01130DERT (AT426320) 46.87 11.92 34.95 PastG202400 0.01130DERT (AT426320) 11.30 28.75 17.45 PastG202400 0.01130DERT (AT426320) 11.30 28.75 17.45 PastG202400 0.01130DERT (AT426326) 0.22 1.67 -1.45 PastG202400 0.0133ANCE (AT5G6426) 0.22 1.63 -1.45 PastG2090500 <td< td=""><td>Psat2d026880</td><td>0.00250</td><td>None</td><td>1.40</td><td>2.90</td><td>-1.50 -49.73</td></td<>	Psat2d026880	0.00250	None	1.40	2.90	-1.50 -49.73
PastEgi9320 0.00383None 13.70 0.96 12.74 PastTy06720 0.00385LPLA12 (AT5G60490) 2.24 22.82 -20.86 PastTy06720 0.00385LPLA12 (AT5G60490) 3.70 9.33 10.36 -9.98 PastTy00720 0.006822CD1 (AT1G49040) 3.70 9.03 -5.33 9.84 PastTy120680 0.006982CD1 (AT1G49040) 45.67 12.16 3.51 PastTy1305120 0.00998CD1 (AT1G2050) 0.00 0.55 -0.55 PastTy1305120 0.00998CD1 (AT1G2050) 0.00 0.05 -0.55 PastTy1305120 0.01170WFKY51 (AT5G644010) 7.38 2.12 5.26 PastTy216420 0.01133MC1 (AT1G02170) 11.30 2.87 -17.46 PastTy21440 0.0133None 2.99 0.00 2.99 2.91 -14.45 PastTy099760 0.01798FL2 (AT5G64260) 0.22 1.67 -1.45 -2.45 PastTy099960 0.01738FK12 (AT5G64260) 0.23 5.416 2.04.17 -1.44 -2.31 -1.44	Psat7g234040	0.00313	None	0.00	9.64	-9.64
Psatr g007520 0.00385PLA11 (A15003170) 2.24 22.82 20.83 Psat054005g0040 0.00520FLA12 (A1560490) 0.38 10.36 -9.98 Psat054005g0040 0.00520FLA12 (A1560490) 3.70 9.03 -5.33 Psat054005g0040 0.006825C10 (A11649040) 3.70 9.03 -5.33 Psat054005g0040 0.00887None 68.35 29.95 38.40 Psat05402820 0.00989RCC11 (A1366350) 16.98 0.00 16.98 Psat1g136120 0.00989RCC11 (A1366350) 0.00 15.95 -1.745 Psat5g228400 0.011310Kr(1 (A15064810) 7.38 2.12 5.26 Psat1g2120 0.01313None 2.99 0.00 2.99 Psat1g099600 0.01775WEL14 (A126390) 5.49 2.392 -18.43 Psat1g015200 0.01865RAP1 (A11643170) 9.10 2.18 3.12.73 Psat1g006240 0.02887None 302.35 41.61 2260.22 Psat1g006240 0.02887None 302.35 41.61 2260.22 Psat1g006240 0.02887None 302.35 41.61 22471.19 <td< td=""><td>Psat6g199320</td><td>0.00383</td><td></td><td>13.70</td><td>0.96</td><td>12.74</td></td<>	Psat6g199320	0.00383		13.70	0.96	12.74
Pastig197440 COUNT ON CONSTRUCT (NO CONSTRUCT) COUNT ON CONSTRUCT COUNT ON CONSTRUCT Pastig197400 COUNT ON CONSTRUCT COUNT ON COU	Psat/g06/920 Psat0s4905d0040	0.00385	FLA11 (A15G03170) FLA12 (AT5G60490)	2.24	22.82	-20.58
Psat7g10082 0.00682SCD1 (AT1G49040) 3.70 9.03 -5.33 Psat6g1093360 0.00698None 68.35 29.95 38.40 Psat6g193360 0.00998CCD1 (AT3G6320) 16.98 0.00 16.98 Psat1g1082820 0.00998CCD1 (AT3G6320) 16.98 0.00 0.55 Psat5g28400 0.01130ER1 (AT4G2930) 46.87 11.92 34.95 Psat5g04800 0.01130ER1 (AT4G2930) 46.87 11.92 34.95 Psat5g04800 0.01133MC1 (AT1G02170) 11.30 28.75 -17.45 Psat1g216120 0.0133AMC1 (AT1G02170) 11.30 28.75 -17.45 Psat7g099760 0.01778MEE14 (AT2G1580) 0.22 1.67 -1.45 Psat4g008520 0.01858ARP1 (AT1G43170) 9.10 21.83 -12.73 Psat6g098600 0.0178MEE14 (AT3G18870) 10.23 6.31 3.92 Psat6g098600 0.02387None 6528.98 22471.19 -15942.21 Psat6g0938600 0.02448Na76G070 16.37 39.92 -2.35	Psat5g197640	0.00610	None	225.85	6.27	219.58
Psat6j13330 0.00689None 68.35 29.95 38.40 Psat6j133300 0.00975Vacuolar iron transporter homolog 2 (AT1G76800) 45.67 12.16 33.51 Psat6j133300 0.0098PFP-ALPHA1 (AT1G2050) 0.00 0.55 -0.55 Psat5g228400 0.01113DER1 (AT4629330) 46.87 11.92 34.95 Psat5g228400 0.0113MC1 (AT1G02170) 7.38 2.12 5.26 Psat1g216120 0.01313None 2.99 0.00 2.99 Psat1g2099760 0.01759EXL2 (AT5G64260) 0.22 1.67 -1.45 Psat1g2099760 0.01759EXL2 (AT5G64260) 0.22 1.67 -1.45 Psat6j008520 0.01859None 302.35 41.61 260.74 Psat6j008520 0.01856Nene 6528.88 22471.19 -15942.21 Psat6g008200 0.02387None 6528.88 22471.19 -15942.21 Psat7g089640 0.02387None 6528.88 22471.19 -15942.21 Psat0303836g0040 0.02414None 0.00 1.59 -0.99	Psat7g009720	0.00682	SCD1 (AT1G49040)	3.70	9.03	-5.33
Psattg98350 0.00993 CCD1 (AT3G6320) 16.000 (a) 12.10 33.31 Psattg9820 0.00998 CCD1 (AT3G6320) 16.08 0.00 .55 -0.55 Psattg22040 0.01136 DERI (AT420950) 0.00 .55 -0.55 Psat5g22400 0.011313 AMC1 (AT1G2170) 11.30 28.75 -17.45 Psat1g216120 0.01313 AMC1 (AT1G02170) 11.30 28.75 -17.45 Psat1g214120 0.01759 EXL2 (ATSG64260) 0.22 1.67 -1.45 Psat7g099960 0.01759 EXL2 (ATSG64260) 0.22 1.63 -12.73 Psat6g006240 0.02285 DGGL (ATSG18570) 10.23 6.31 3.92 Psat6g006240 0.02387 None 6528.98 22471.19 -15942.21 Psat6g0023960 0.02469 ATSG20670 16.37 3.9.92 -2.3.55 Psat6g003800 0.0270 PCR2 (AT1G14870) 4.477 6.74 38.64 Psat6g03800 0.0270 PCR2 (AT1G14870) 4.477 6.74 38.04 Psat6g1203900 0.02660 ATSG20670 16.37 3.9.92	Psat7g120680	0.00689	None	68.35	29.95	38.40
Psattg138120 0.00998PFP-ALPHA1 (AT1620950) 0.00 0.55 -0.55 Psat5g228400 0.01136DER1 (AT4629330) 46.87 11.92 34.95 Psat5g064800 0.01133AMC1 (AT1602170) 11.30 28.75 -17.45 Psat1g0216120 0.01313None 2.99 0.00 2.99 Psat7g099760 0.01778MEE14 (AT2664260) 0.22 1.67 -1.45 Psat7g099960 0.01778MEE14 (AT2615890) 5.49 23.92 -18.43 Psat6g192200 0.01865ARP1 (AT1643170) 9.10 21.83 -12.73 Psat6g006240 0.02058 OEGL (AT5618570) 10.23 6.31 3.92 Psat7g0998040 0.02414None 0.00 3.98 -3.98 Psat6g023960 0.02478None 6528.98 22471.19 -15942.21 Psat6g023960 0.02476ATS6070 16.37 39.92 -23.55 Psat6g023960 0.02469ATS620670 16.37 39.92 -23.55 Psat6g023960 0.022092PSBA (ATC600020) 55.98 122.47 -66.49	Psat1q098280	0.00998	CCD1 (AT3G63520)	16.98	0.00	16.98
Psat5g04800 0.01130DERI (AT4G29330) 48.87 11.92 34.95 Psat5g064800 0.01170VRKY51 (AT5G64810) 7.38 2.12 5.26 Psat1g221440 0.01313AMC1 (AT1G02170) 11.30 28.75 -17.45 Psat7g099960 0.01759EXL2 (AT5G64260) 0.22 1.67 -14.45 Psat4g008520 0.01859None 302.35 41.61 260.74 Psat6g102200 0.01865ARP1 (AT1G43170) 9.10 21.83 -12.73 Psat7g089640 0.02387ABCG11 (AT1G43170) 9.10 21.83 -12.73 Psat6g006240 0.020387ABCG11 (AT1G17840) 6528.98 22471.19 -15942.21 Psat6g023960 0.02414None 0.00 3.98 -3.98 Psat6g023960 0.0240AT5620670 16.37 39.92 -2.35 Psat5g098320 0.02666ATMRP11 (AT2G07680) 0.60 1.59 -0.99 Psat5g098320 0.02586ATMRP11 (AT2G07680) 2.17 4.48 -2.31 Psat5g098320 0.02566ATMRP11 (AT2G07680) 55.20 23.53 31.67 Psat5g190320 0.02566ATMRP11 (AT2G07680) 55.20 23	Psat1g136120	0.00998	PFP-ALPHA1 (AT1G20950)	0.00	0.55	-0.55
Psatb3064800 0.01170WKRY51 (A15G64810) 7.38 2.12 5.26 Psatb3216120 0.01313MC1 (AT1602170) 11.30 28.75 -17.45 Psatb32099700 0.01759EXL2 (AT5G64260) 0.22 1.67 -1.45 Psatb3099800 0.01778MEE14 (AT2G15890) 3.29 -18.43 -12.73 Psatb3006240 0.020580BGL (AT5G18570) 9.10 21.83 -12.73 Psatb306240 0.02387None 6528.98 22471.19 -15942.21 Psatb306240 0.02387None 6528.98 22471.19 -15942.21 Psatb309300 0.02469AT5G20670 16.37 39.92 -23.55 Psatb309300 0.0256ATMRP11 (AT2G07680) 0.60 1.59 -0.99 Psatb309300 0.0292PSBA (ATG600020) 55.98 122.47 -66.49 Psatb31050800 0.03079AT3G01660 55.20 23.53 3.61 -2.39 Psatb31050800 0.03261ATG522450 0.73 0.15 0.58 Psatb310530080 0.0329AT3G01660 3.91 15.80 -11.89 Psatb3153058520 0.03261AT5G24430 3.64 0.99	Psat5g228400	0.01136	DER1 (AT4G29330)	46.87	11.92	34.95
Psat1g221440 0.01313None 2.99 0.00 2.99 Psat7g099760 0.01778PKL2 (AT5G64260) 0.22 1.67 -1.45 Psat7g099960 0.0178WEE14 (AT2G15890) 5.49 23.92 -18.43 Psat4g008520 0.01865ARP1 (AT1G43170) 9.10 21.83 -12.73 Psat6g006240 0.020387ABCG11 (AT1G43170) 9.10 21.83 -12.73 Psat7g089640 0.02387None 6528.98 22471.19 -15942.21 Psat6g023060 0.02414None 0.00 3.98 -3.98 Psat6g023060 0.0249AT5G20670 16.37 3.99.2 -2.85 Psat6g023060 0.02702PCR2 (AT1G14870) 44.77 6.74 38.04 Psat6g023060 0.02702PCR2 (AT1G14870) 44.77 6.74 38.04 Psat6g168240 0.0392PSBA (ATC600020) 55.98 12.247 -66.49 Psat0s9163g0080 0.03109None 4.27 12.50 -8.22 Psat6g168240 0.0323AT1G34340 3.64 0.99 2.65 Psat6g168240 <td>Psat3g064800 Psat1g216120</td> <td>0.01170</td> <td>AMC1 (AT1G02170)</td> <td>11.30</td> <td>2.12</td> <td>5.20 -17 45</td>	Psat3g064800 Psat1g216120	0.01170	AMC1 (AT1G02170)	11.30	2.12	5.20 -17 45
Psat7g099760 0.01759EXL2 (AT5G64260) 0.22 1.67 -1.45 Psat7g099960 0.01778MEE14 (AT2G15890) 5.49 23.92 -18.43 Psat6g008520 0.01859None 302.35 41.61 260.74 Psat6g008520 0.01865ARP1 (AT1G43170) 9.10 21.83 -12.73 Psat6g08940 0.02387None 6528.98 22471.19 -15942.21 Psat7g08960 0.02487ABCG11 (AT1G17840) 6528.98 22471.19 -15942.21 Psat6g023960 0.02469AT5620670 16.37 39.92 -23.55 Psat5g098320 0.02566ATMRP11 (AT2G07680) 0.60 1.59 -0.99 Psat47g00360 0.02702PCR2 (AT1G14870) 44.77 6.74 38.04 Psat0323960 0.02932LL4 (AT1G51760) 2.17 4.48 -2.31 Psat43900360 0.02702PCR2 (AT1G14870) 45.98 12.247 -66.49 Psat039163600 0.02992PSBA (ATCG00020) 55.98 12.247 -66.49 Psat0391630080 0.03109None 4.27 12.50 -8.22 <td>Psat1g221440</td> <td>0.01313</td> <td>None</td> <td>2.99</td> <td>0.00</td> <td>2.99</td>	Psat1g221440	0.01313	None	2.99	0.00	2.99
Psat/g009960 0.01778MEE14 (A12G15890) 5.49 23.92 -18.43 Psat6g192200 0.018659None 302.35 41.61 260.74 Psat6g192200 0.01865ARP1 (AT1G43170) 9.10 21.83 -12.73 Psat6g192200 0.020580BGL (AT5G18570) 10.23 6.31 3.92 Psat7g089640 0.02387None 6528.98 22471.19 -15942.21 Psat0s3396g0040 0.02414None 0.00 3.98 -3.98 Psat5g023960 0.02469AT5G20670 16.37 39.92 -23.55 Psat5g093820 0.02566ATMRP11 (AT2G07680) 0.60 1.59 -0.99 Psat4g126840 0.0292PSBA (ATCG0020) 44.77 6.74 38.04 Psat0s3967g0160 0.0292PSBA (ATCG00020) 55.98 122.47 -66.49 Psat0s9163g0080 0.03109None 4.27 12.50 -8.22 Psat6g186240 0.03261AT5G2450 0.73 0.15 0.58 Psat6g186240 0.03261AT2G3430 0.02 0.10 -0.08 Psat6g194200 0.03261AT2G34930 0.02 0.10 -0.08 <	Psat7g099760	0.01759	EXL2 (AT5G64260)	0.22	1.67	-1.45
Datageoch Distortion Distortion Distortion Distortion Psat6g192200 0.01865ARP1 (AT1G43170) 9.10 21.83 -12.73 Psat6g006240 0.020580BGL (AT5G18570) 10.23 6.31 3.92 Psat7g089640 0.02387ABCG11 (AT1G17840) 6528.98 22471.19 -15942.21 Psat0s3396g0040 0.02469AT5620670 16.37 39.92 -23.55 Psat5g093830 0.02566ATMRP11 (AT2G07680) 0.60 1.59 -0.99 Psat4g126840 0.02952ILL4 (AT1G51760) 2.17 4.48 -2.31 Psat0s38367g0160 0.023292PSBA (ATCG00020) 55.98 122.47 -66.49 Psat0s163g0080 0.03109None 4.27 12.50 -8.22 Psat0s163g0080 0.03261AT3G2450 0.73 0.15 0.58 Psat6g194200 0.03261AT6G2450 0.73 0.15 0.58 Psat6g194200 0.03261AT2G34930 0.00 0.63 -0.62 Psat6g194200 0.03261AT2G34930 0.00 0.63 -0.62	Psat/g099960 Psat4q008520	0.01778	MEE14 (AT2G15890) None	5.49 302 35	23.92	-18.43
Psatbg006240 0.02058 OBGL (AT5G18570) 10.23 6.31 3.92 Psatf g089640 0.02387 None 6528.98 22471.19 -15942.21 Psatf g091320 0.02387 ABCG11 (AT1G17840) 6528.98 22471.19 -15942.21 Psatf g09360 0.02469 AT5G20670 16.37 39.92 -23.55 Psatf g009360 0.02702 PCR2 (AT1G14870) 44.77 6.74 38.09 Psatf g009360 0.02952 ILL4 (AT1G51760) 2.17 4.48 -2.31 Psatd g10640 0.02992 PSBA (ATCG00020) 55.98 122.47 -66.49 Psatd g10640 0.03203 AT1G34340 3.64 0.99 2.65 Psatd g00800 0.03261 AT5G22450 3.51 3.51 0.58 Psat5g058520 0.03261 AT5G22450 0.73 0.15 0.58 Psat5g19500 16.70 27.58 -10.89 Psat5g191320 0.03803 ERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat5g191320 0.03863 YLS7 (TBL17, AT5G51640) 2.26 5.50 -3.25 Psat5g19	Psat6g192200	0.01865	ARP1 (AT1G43170)	9.10	21.83	-12.73
Psat7g089640 0.02387None 6528.98 22471.19 -15942.21 Psat7g091320 0.02387ABCG11 (AT1G17840) 6528.98 22471.19 -15942.21 Psat0s396g0040 0.02414None 0.00 3.98 -3.98 Psat6g023960 0.02469AT5G20670 16.37 39.92 -23.55 Psat5g098320 0.02566ATMRP11 (AT2G07680) 0.60 1.59 0.99 Psat4g108640 0.0292PCR2 (AT1G14870) 44.77 6.74 38.04 Psat4g126840 0.02929LL4 (AT1651760) 2.17 4.48 -2.31 Psat0s3367g0160 0.03079AT3G01660 55.20 23.53 31.67 Psat6g186240 0.0323AT1G34340 3.64 0.99 2.65 Psat5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat5g186240 0.03261AT5G22450 0.02 0.10 -0.08 Psat5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat5g191320 0.03603SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat5g191320 <td>Psat6g006240</td> <td>0.02058</td> <td>OBGL (AT5G18570)</td> <td>10.23</td> <td>6.31</td> <td>3.92</td>	Psat6g006240	0.02058	OBGL (AT5G18570)	10.23	6.31	3.92
Psatb 3396 0.02337 ABCGTT (ATTGT7840) 0.522.96 22471.19 -15942.21 Psatb 3396g0040 0.02414None 0.00 3.98 -3.38 Psatb 3396g0040 0.02469AT5G20670 16.37 39.92 -23.55 Psatb 3390 0.02702 PCR2 (AT1G17860) 0.60 1.59 0.99 Psatb 3390 0.02702 PCR2 (AT1G14870) 44.77 6.74 38.04 Psatb 32640 0.0292 PSBA (ATCG00020) 55.98 122.47 -66.49 Psatb 33670160 0.03079 AT3G01660 55.20 23.53 31.67 Psatb 320080 0.03109 None 4.27 12.50 -8.22 Psatb 32026840 0.0323 AT1G34340 3.64 0.99 2.65 Psatb 32058520 0.03261 AT5G22450 0.73 0.15 0.58 Psatb 32040 0.03261 AT5G2450 0.02 0.10 -0.08 Psatb 320304 0.03261 AT5G2450 0.63 -0.62 -1.89 Psatb 3213040 0.03325 AT3G51950 16.70 27.58 -10.89 Psatb 32040	Psat7g089640	0.02387		6528.98	22471.19	-15942.21
Psat6g023960 0.02469AT5G20670 16.37 39.92 -23.55 Psat5g098320 0.02566ATMRP11 (AT2G07680) 0.60 1.59 0.99 Psat7g009360 0.02702PCR2 (AT1G14870) 44.77 6.74 38.04 Psat4g126840 0.0292PCR2 (AT1G14870) 2.17 4.48 -2.31 Psat0ss367g0160 0.02992PSBA (ATCG00020) 55.98 122.47 -66.49 Psat0ss367g0160 0.03079AT3G01660 55.20 23.53 31.67 Psat0ss367g0180 0.03109None 4.27 12.50 -8.22 Psat6g186240 0.0323AT1G34340 3.64 0.99 2.65 Psat5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat5g194200 0.03261AT5G22450 0.73 0.15 0.58 Psat5g191320 0.03603SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat5g191320 0.0386None 12.50 -3.25 -3.25 Psat5g191320 0.0386None 12.50 0.00 12.50 Psat1g005120 0.0	Psat0s3396g0040	0.02387	None	0.00	3.98	-13942.21
Psat5g098320 0.02566ATMRP11 (AT2G07680) 0.60 1.59 -0.99 Psat7g009360 0.02702PCR2 (AT1G14870) 44.77 6.74 38.04 Psat4g126840 0.02952ILL4 (AT1G51760) 2.17 4.48 -2.31 Psat0ss367g0160 0.02992PSBA (ATCG00020) 55.98 122.47 -66.49 Psat0ss3163g0080 0.03109None 4.27 12.50 -8.22 Psat6g186240 0.0323AT1G34340 3.64 0.99 2.65 Psat5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat5g191320 0.03261AT5G22450 0.73 0.15 0.58 Psat5g191320 0.03261AT5G22450 0.02 0.10 -0.08 Psat5g191320 0.03261AT5G2450 0.73 0.15 0.58 Psat5g191320 0.03603SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat5g191320 0.0386None 12.50 -3.25 -3.25 Psat5g05120 0.0386None 12.50 0.00 12.50 Psat5g05120 0.03886None<	Psat6g023960	0.02469	AT5G20670	16.37	39.92	-23.55
Psatrg009360 0.02702PCR2 (A11614870) 44.77 6.74 38.04 Psatdg126840 0.02952ILL4 (A1161760) 2.17 4.48 -2.31 Psatdsg126840 0.02992PSBA (ATCG00020) 55.98 122.47 -66.49 Psatdsg171640 0.03079AT3G01660 55.20 23.53 31.67 Psat0sg163g0080 0.03109None 4.27 12.50 -8.22 Psat6g186240 0.03233AT1G34340 3.64 0.99 2.65 Psat5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat5g194200 0.03261AT5G2450 0.02 0.10 -0.08 Psat5g191320 0.03603SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat1g111400 0.03863YLS7 (TBL17, AT5G51640) 2.26 5.50 -3.25 Psat5g105120 0.03868None 12.50 0.00 12.50 Psat5g124680 0.04654PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat5g098320	0.02566	ATMRP11 (AT2G07680)	0.60	1.59	-0.99
Distribution Distribution<	Psat/g009360 Psat4g126840	0.02702	PCR2 (AT1G14870) III 4 (AT1G51760)	44.77	6.74 4.48	38.04 -2.31
Psat3g171640 0.03079AT3G01660 55.20 23.53 31.67 Psat0s9163g0080 0.03109None 4.27 12.50 -8.22 Psat6g186240 0.03233AT1G34340 3.64 0.99 2.65 Psat5g026840 0.03261None 3.91 15.80 -11.89 Psat5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat5g058520 0.03261AT5G22450 0.02 0.10 -0.08 Psat5g058520 0.03261AT5G2450 0.02 0.10 -0.08 Psat5g194200 0.03263AT3G51950 16.70 27.58 -10.89 Psat5g191320 0.03603SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat1g111400 0.03863YLS7 (TBL17, AT5G51640) 2.26 5.50 -3.25 Psat6g054320 0.03868None 12.50 0.00 12.50 Psat1g005120 0.03886None 12.50 0.00 12.50 Psat4g086600 0.04426MAPKKK21 (AT4G36950) 1.52 7.08 -5.56 Psat5g124680 0.04654PP2CA (AT3G1	Psat0ss8367g0160	0.02992	PSBA (ATCG00020)	55.98	122.47	-66.49
Psatts9163g0080 0.03109None 4.27 12.50 -8.22 Psatt6g186240 0.03233AT1G34340 3.64 0.99 2.65 Psatt6g186240 0.03233AT1G34340 3.91 15.80 -11.89 Psatt5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psatt5g058520 0.03261AT2G34930 0.02 0.10 -0.08 Psatt5g194200 0.03261AT2G34930 0.02 0.10 -0.08 Psatt5g191320 0.03603SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psatt9g191320 0.03683YLS7 (TBL17, AT5G51640) 2.26 5.50 -3.25 Psatt9g054320 0.03868None 12.50 0.00 12.50 Psatt9g086600 0.04426MAPKKK21 (AT4G36950) 1.52 7.08 -5.56 Psatt9g1268600 0.04654PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat3g171640	0.03079	AT3G01660	55.20	23.53	31.67
Psat2g026840 0.03261Nnoe 3.91 15.80 -11.89 Psat2g0268520 0.03261AT5G22450 0.73 0.15 0.58 Psat6g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat5g058520 0.03261AT5G22450 0.02 0.10 -0.08 Psat5g194200 0.03261AT2G34930 0.02 0.10 -0.08 Psat5g191320 0.03603SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat6g054320 0.03852F9H_3 (AT4G03420) 2.26 5.50 -3.25 Psat6g054320 0.03852F9H_3 (AT4G03420) 0.40 1.73 -1.33 Psat1g005120 0.03886None 12.50 0.00 12.50 Psat4g086600 0.04426MAPKKK21 (AT4G36950) 1.52 7.08 -5.56 Psat5g124680 0.04654PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat0s9163g0080 Psat6g186240	0.03109	None AT1G34340	4.27	12.50	-8.22
Psat5g058520 0.03261AT5G22450 0.73 0.15 0.58 Psat6g194200 0.03261AT2G34930 0.02 0.10 -0.08 Psat5g213040 0.0325AT3G51950 16.70 27.58 -10.89 Psat5g191320 0.0303SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat1g111400 0.03852F9H_3 (AT4G03420) 2.26 5.50 -3.25 Psat6g054320 0.03852F9H_3 (AT4G03420) 0.40 1.73 -1.33 Psat1g005120 0.03886None 12.50 0.00 12.50 Psat5g124680 0.04654PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat2g026840	0.03261	None	3.91	15.80	-11.89
Psatt6g194200 0.03261AT2G34930 0.02 0.10 -0.08 Psat5g213040 0.0325AT3G51950 16.70 27.58 -10.89 Psat5g191320 0.03603SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat1g111400 0.03852F9H_3 (AT4G03420) 2.26 5.50 -3.25 Psat1g005120 0.03886None 12.50 0.00 12.50 Psat5g026600 0.04426MAPKKK21 (AT4G36950) 1.52 7.08 -5.56 Psat5g124680 0.04654PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat5g058520	0.03261	AT5G22450	0.73	0.15	0.58
Psat5g191320 0.03603 SERGT1 (AT3G01720) 0.00 0.63 -0.62 Psat1g111400 0.03683 YLS7 (TBL17, AT5G51640) 2.26 5.50 -3.25 Psat6g054320 0.03852 F9H_3 (AT4G03420) 0.40 1.73 -1.33 Psat1g005120 0.03886 None 12.50 0.00 12.50 Psat6g06600 0.04426 MAPKKK21 (AT4G36950) 1.52 7.08 -5.56 Psat5g124680 0.04654 PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat6g194200 Psat5g213040	0.03261	A12G34930 AT3G51950	0.02	0.10	-0.08
Psat1g111400 0.03683YLS7 (TBL17, AT5651640) 2.26 5.50 -3.25 Psat6g054320 0.03852F9H_3 (AT4G03420) 0.40 1.73 -1.33 Psat1g005120 0.03886None 12.50 0.00 12.50 Psat4g086600 0.04426MAPKKK21 (AT4G36950) 1.52 7.08 -5.56 Psat5g124680 0.04654PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat5g191320	0.03603	SERGT1 (AT3G01720)	0.00	27.58	-10.89
Psat6g054320 0.03852F9H_3 (AT4G03420) 0.40 1.73 -1.33 Psat1g005120 0.03886None 12.50 0.00 12.50 Psat4g086600 0.04426MAPKKK21 (AT4G36950) 1.52 7.08 -5.56 Psat5g124680 0.04654PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat1g111400	0.03683	YLS7 (TBL17, AT5G51640)	2.26	5.50	-3.25
Psactguosizo 0.03886 None 12.50 0.00 12.50 Psat4g086600 0.04426 MAPKK21 (AT4G36950) 1.52 7.08 -5.56 Psat5g124680 0.04654 PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat6g054320	0.03852	F9H_3 (AT4G03420)	0.40	1.73	-1.33
Psat5g124680 0.04654PP2CA (AT3G11410) 3.35 7.86 -4.51	Psat1g005120 Psat4g086600	0.03886	NORE MAPKK21 (AT4G36950)	12.50	0.00	12.50
	Psat5g124680	0.04654	PP2CA (AT3G11410)	3.35	7.86	-4.51

Primer name	Sequence	Purpose
AtBTS-F	GCTATTCTAACCGTGAGA	Amplify a region encompassing the <i>bts-3</i>
AtBTS-R	AGAACCACAGCTACCAC	mutation, followed by PfIMI digestion, which cleaves WT but not the <i>bts</i> -3 allele.
JBSH167	GCGTGAAGAATGTAGCACAG	Detect the pea dgl allele based on PCR
JBSH168	ACCTGCAATATTCAACCAGCA	product length difference
M95	GTCCCTCCTTCTAACTCCG	Detect the <i>AtBTS</i> transgene in Arabidopsis
M103	TCCTTAGCCATGTGTTGACT	
M95	GTCCCTCCTTCTAACTCCG	Detect the <i>PsBTS1</i> transgene in
M105	TTCATCAGTTTCCGTGGC	Arabidopsis
M104	CCTTAGCCATGTGCTGC	Detect the <i>BTS1^{dg/}</i> transgene in Arabidopsis
JBSH190	ACGAGCTAGATGCGTTGCACCG	
MtOPT3-F1	CTTAGTCCTCTCTCGCATTG	TILLING of Medicago OPT3 target region
MtOPT3-R3	ACACCCATAAAAGCTGTG	
MtBTS1-F1	ATAGTCCGGTCTTCCTGTGC	TILLING of Medicago <i>BTS1</i> target region
MtBTS1-R3	GCCTTGCATTCACCACTATAG	
PsOPT3-F4	GACATATTGAGACGAGCAGG	Detect the pea <i>brz</i> mutation by PCR and
PsOPT3-R5	ATACCCAATCATGAACTGTGC	Sanger sequencing

Supplemental Table 3 - Primers used in this study.

Supplemental Table 4 - TILLING mutations in Medicago truncatula genes.

Medicago <i>BTS1 /</i> <i>Medtr6g083900</i>	Amino acid change	Phenoptype in Medicago
c.G2188A	p.Asp730Asn	Embryo lethal
c.G2419A	p.Gly807Arg	None*
c.G2734A	p.Asp912Asn	None
c.G2800A	p.Leu934Phe	None
Medicago OPT3 / Medtr4g133968		
c.G1219A	p.Asp407Asn	None
c.C1586T	p.Pro529Leu	Iron accumulation, bronze spots on older leaves, impaired growth