Phenotypic evaluation and QTL analysis of yield and symbiotic nitrogen fixation in a common bean population grown with two levels of phosphorus supply

Citation:

Diaz, Lucy M.; Ricaurte, Jaumer; Cajiao, Cesar; Galeano, Carlos H.; Rao, Idupulapati; Beebe, Steve; Raatz, Bodo. 2017. Phenotypic evaluation and QTL analysis of yield and symbiotic nitrogen fixation in a common bean population grown with two levels of phosphorus supply. Molecular Breeding 37: 76.

Publisher's DOI:
http://dx.doi.org/10.1007/s11032-017-0673-1

Access through CIAT Research Online:
http://hdl.handle.net/10568/81384

Molecular Breeding

Phenotypic evaluation and QTL analysis of yield and symbiotic nitrogen fixation in a common bean population grown with two levels of phosphorus supply

--Manuscript Draft--

Manuscript Number:	MOLB-D-16-00386
Full Title:	Phenotypic evaluation and QTL analysis of yield and symbiotic nitrogen fixation in a common bean population grown with two levels of phosphorus supply
Article Type:	Original Article
Keywords:	Phaseolus vulgaris; symbiotic nitrogen fixation; yield components
Corresponding Author:	Bodo Raatz Centro Internacional de Agricultura Tropical Palmira, COLOMBIA
Corresponding Author Secondary Information:	
Corresponding Author's Institution:	Centro Internacional de Agricultura Tropical
Corresponding Author's Secondary Institution:	Centro Internacional de Agricultura Tropical
First Author:	LUCY DIAZ
First Author Secondary Information:	
Order of Authors:	LUCY DIAZ
	Jaumer Ricaurte
	Cesar Cajiao
	Carlos Galeano
	Idupulapati Rao
	Steve Beebe
	Bodo Raatz
Order of Authors Secondary Information:	
Funding Information:	
Abstract:	Common bean is an important staple crop in Eastern Africa and Latin America. Low soil fertility is a major limitation to agronomic productivity. Symbiotic nitrogen fixation (SNF) is an important property of legumes, leading to high protein levels and high nutritional value. SNF and yield traits were evaluated in the common bean population DOR 364 × BAT 477 in field experiments under moderate and low phosphorus (P) soil conditions resembling environments found on farmer's fields. Low P availability in soil severely limits grain yield, and trait correlations with yield reveal that high biomass as well as early maturity and efficient seed filling are important for good performance in low P stress, resembling drought resistance. Investigation of SNF related traits under low P stress showed reduced seed nitrogen levels, but no significant reduction of %N derived from atmosphere (%Ndfa), however %Ndfa was correlated with yield in low P conditions, indicating that under stress SNF becomes an important asset. Significant genetic variation as well as transgressive segregation was observed for yield, yield components and SNF ability suggesting that traits can be improved by breeding. QTLs for %Ndfa and seed N concentration were discovered on chromosomes Pv07 and Pv02, independent yield QTLs were identified on the same chromosomes. Two QTL hotspots that affect several traits including yield components were found on Pv02

	and Pv06, the latter represents a constitutive QTL hotspot independent from the environment. QTLs may be used for marker design and molecular breeding.
Suggested Reviewers:	Karen Cichy Michigan State University cichykar@msu.edu Improving the nitrogen fixation capacity of dry beanDevelopment of improved bean germplasm
	James Kelly Michigan State University kellyj@msu.edu Plant and Soil Sciences BuildingDevelopment of improved bean germplasmImproving the nitrogen fixation capacity of dry bean
	Juan Carlos Rosas jcrosas@zamorano.edu Development of improved bean germplasm

Phenotypic evaluation and QTL analysis of yield and symbiotic nitrogen fixation in a common bean population grown with two levels of phosphorus supply

Lucy M. Diaz[1], Jaumer Ricaurte[1], Cesar Cajiao[1], Carlos H. Galeano[2], Idupulapati Rao[1], Steve Beebe[1], Bodo Raatz[1]

(1) International Center for Tropical Agriculture, Biotechnology Unit and Bean Program, A. A. 6713, Cali, Valle, Colombia.

(2) Corporación Colombiana de investigación Agropecuaria (Corpoica), Km 14 vía Mosquera, Bogotá, Colombia.

Received:

Corresponding author: ✉ B. Raatz

CIAT - International Center for Tropical Agriculture

A. A. 6713, Cali, Colombia, South América

Tel: 57-2-4450-000; VIA USA 650-833-6625

Fax: 57-2-4450-073; VIA USA 650-833-6626 or 305-592-9757

E-mail: b.raatz@cgiar.org

Keywords: *Phaseolus vulgaris*, symbiotic nitrogen fixation, yield components

Abbreviations:

100SDW	100 seed weight
CID	Carbon isotope discrimination in grain
SDCN	Carbon to nitrogen ratio in seed
DF	Days to flowering
DPM	Days to physiological maturity
Ndfs_ha	Nitrogen derived from soil per ha
Ndfa_ha	Nitrogen derived from the atmosphere per ha
%Ndfa	Percentage of nitrogen derived from the atmosphere
FVFM	Photosynthetic efficiency on younger fully expanded leaf
LP	Low phosphorus stress
MP	Moderate phosphorus
PBH	Pod biomass at harvest
PHI	Pod harvest index
PNA	Pod number per area
QTL	Quantitative trait locus
RIL	Recombinant inbred line
SDC	Seed carbon content in per cent
SDN	Seed nitrogen content in per cent
SDN_ha	Seed nitrogen per ha
SDNA	Seed number per area
SHBH	Shoot biomass at harvest
SCMR	SPAD chlorophyll meter reading in younger fully expanded leaf
SBH	Stem biomass at harvest
SCOND	Stomatal conductance on younger fully expanded leaf

YDHA Yield per hectare

Author Contribution Statement

Diaz LM: Writing of the manuscript, analysis of genetic data and statistical analysis of phenotypic data

Ricaurte J: Field evaluation and statistical analysis of phenotypic data

Cajiao C: Trial design and field evaluation

Galeano C: Idea conception and preliminary analysis of linkage and QTL.

Rao I: leadership of physiological evaluations, discussion and improvements of the manuscript

Beebe S: Idea conception, leadership of field trial activities, discussion and improvements of the manuscript

Raatz B: leadership of genetic analysis, writing of the manuscript

Key Message

Low Phosphorous stress in *Phaseolus vulgaris* reduces yield and seed nitrogen, but has no strong effect on % nitrogen derived from atmosphere. QTLs were identified for SNF and yield components.

Abstract

Common bean is an important staple crop in Eastern Africa and Latin America. Low soil fertility is a major limitation to agronomic productivity. Symbiotic nitrogen fixation (SNF) is an important property of legumes, leading to high protein levels and high nutritional value.

SNF and yield traits were evaluated in the common bean population DOR 364 × BAT 477 in field experiments under moderate and low phosphorus (P) soil conditions resembling environments found on farmer's fields. Low P availability in soil severely limits grain yield, and trait correlations with yield reveal that high biomass as well as early maturity and efficient seed filling are important for good performance in low P stress, resembling drought resistance.

Investigation of SNF related traits under low P stress showed reduced seed nitrogen levels, but no significant reduction of %N derived from atmosphere (%Ndfa), however %Ndfa was correlated with yield in low P conditions, indicating that under stress SNF becomes an important asset. Significant genetic variation as well as transgressive segregation was observed for yield, yield components and SNF ability suggesting that traits can be improved by breeding.

QTLs for %Ndfa and seed N concentration were discovered on chromosomes Pv07 and Pv02, independent yield QTLs were identified on the same chromosomes. Two QTL hotspots that affect several traits including yield components were found on Pv02 and Pv06, the latter represents a constitutive QTL hotspot independent from the environment. QTLs may be used for marker design and molecular breeding.

Introduction

Common bean (*Phaseolus vulgaris* L.) is the most important grain legume for direct human consumption and a major source of protein and micronutrients in the tropics (Broughton et al. 2003). Total world production is estimated at around 12 million tons per year (Beebe et al. 2013), a large proportion by resource-limited smallholder farmers.

Phosphorus (P) is an essential macronutrient, adequate supply of P is required for optimal plant growth and development. Approximately 67% of globally cultivated lands have P deficits (Batjes 1997), 50% of the common bean production area worldwide is estimated to be affected by low P stress (Beebe 2012) while in Latin America 60% of beans are estimated to be grown on P-deficit soils (Rao 2014). P in the soil is only partly soluble and not very mobile, therefore plants can only utilize a small fraction of total P in soil (Batjes 2011). Soil P availability is particularly low in strongly acidic or alkaline soils, mainly due to formation of phosphate complexes with Al and Fe in acid soils and Ca complexes in alkaline soils (Marschner 1995). Numerous studies have been carried out to identify beans adapted to low P and acid soils (Lynch and Beebe 1995; Beebe et al. 2008; Cichy et al. 2009a; Cichy et al. 2009b; Ramaekers et al. 2010; Rao 2014). In response to low P availability, common bean modifies its root architecture, associates with mycorrhizal fungi in its root system, and presents a higher efficiency of utilizing absorbed P to produce biomass and grain yield (Beebe et al. 2006; Cichy et al. 2009b; Ramaekers et al. 2010; Rao et al. 2016).

Another essential nutrient for plant growth is nitrogen (N), present in high abundance in the atmosphere and in low levels in most soils. Similar to P, it is a factor limiting growth and yield of crops. Legumes such as common bean are able to fix atmospheric N by symbiotic nitrogen

fixation (SNF). *Rhizobia* which reside in nodules in the roots reduce atmospheric N_2 to ammonium, which is distributed throughout the plant (Araújo et al. 2015).

Various isotope analysis methods have been used to study plant metabolic processes such as carbon isotope discrimination (CID, $\Delta^{13}C$) (Hall et al. 1994; Polania et al. 2016b) and ^{15}N natural abundance ($\delta^{15}N$), (Andrews and Lea 2013). ^{13}C and ^{12}C are present in the biosphere naturally at 1.1 % and 98.9 %, respectively. Plants may discriminate against the heavier isotope $\delta^{13}C$ at the Calvin cycle depending on stomatal activity, which leads to a depletion of the plant dry matter in ^{13}C. Different studies have demonstrated that CID is correlated with water use efficiency (WUE) allowing to identify indirectly genotypes that tolerate water limited conditions (Dhanapal et al. 2015; Polania et al. 2016a). This trait showed stability across environments and high broad sense heritability (Dhanapal et al. 2015) . The $\delta^{15}N$ isotope method is used to quantify the percentage of N derived from the atmosphere (%Ndfa) as ^{15}N is present at a higher proportion in the biosphere compared to the atmosphere (Polania et al. 2016b). N fixation indicators that have been reported in common bean are lateral root nodules, number of nodules, plant biomass, total plant N and grain yield (Bliss 1993).

Studies have been performed to investigate the molecular basis of tolerance to stresses including drought and low soil P (Ramírez et al. 2013) and SNF. The recombinant inbred line (RIL) population of DOR 364 × BAT 477 has been studied by different authors (Galeano et al. 2011; Asfaw and Blair 2012; Blair et al. 2012a; Asfaw et al. 2012) investigating drought, identifying QTLs for yield components, rooting pattern traits and photosynthate remobilization traits. Common bean genotype BAT 477 has been demonstrated to present superior SNF under both optimal and suboptimal conditions, including P stress and drought stress, while genotype DOR 364 has a contrasting responses to low P stress (Ramírez et al. 2013). First QTL mapping

in this population was described by Blair et al. (2010) and Galeano et al. (2011) further improved the genetic map.

In the present study the common bean RIL population of DOR 364 × BAT 477 was evaluated for its response to two levels of soil P supply (moderate and low) at Quilichao, Colombia. A number of traits related to N fixation, yield components, photosynthesis and phenology were evaluated to investigate trait correlations and low P stress effects. Furthermore this study aimed at identifying QTL for major breeding traits to aid the bean breeding program to develop markers for Marker Assisted Selection (MAS).

Materials and methods

Plant materials

A set of 98 Recombinant Inbred Lines (RIL) of the DOR 364 × BAT 477 (D×B) population of common bean (Galeano et al. 2011) was evaluated in this study together with two parents. RILs were obtained from F_5 lines advanced by single seed descent. DOR 364 is a commercial type characterized by small red seeds that shows tolerance to bean golden yellow mosaic virus and has good yield potential in environments with high soil P, but is susceptible to drought. On the other hand, BAT 477 is a small cream-seeded type, tolerant to low P and drought and has high SNF capacity (Sponchiado et al. 1989; Remans et al. 2007; Polanía et al. 2009; Ramírez et al. 2013; Beebe et al. 2013). Both parental lines are from the Mesoamerican genepool.

153 *Experimental design and field conditions*

154 D×B population was planted at CIAT Experimental Station located in Quilichao in Colombia
155 (November 2012 to January 2013) with three replications in a "10x10" lattice experimental
156 design. Seedlings were inoculated using drench method with *Rhizobium tropici* strain CIAT
157 899 (CIAT 1988) at 10 days after sowing. This strain is characterized by a high symbiotic
158 stability and efficient N fixation. Two rows of headers were planted with DOR 364NN (non-
159 nodulating) and BAT 477NN (non-nodulating) and inoculated with the same strain of
160 *Rhizobium*. Plots consisted of two 3.72 m long rows at a 0.6 m row to row distance, planted
161 with 12-15 seeds per m of row length. Nutrients were applied over sown row: 100 kg ha^{-1}
162 $MgSO_4$ and 50 kg ha^{-1} of macro and micronutrients mix as Agrimins (Colinagro, Puerto
163 Tejada, Colombia) in %: 13.0 Ca, 8.0 N, 3.6 Mg, 2.5 Zn, 2.2 P, 1.6 S, 1 B, 0.14 Cu, and 0.005
164 Mo. Low P and moderate P levels in soil were established by applying 10 kg P ha^{-1} and 30 kg
165 P ha^{-1} as Granufos 40, (Productos Químicos Panamericanos, Medellin, Colombia), in %: 17.5
166 P, 19.0 Ca and 3.0 S, respectively. Available soil P (µg g^{-1}, Bray II) was measured at 0-5, 5-
167 10, 10-20, 20-40 cm soil depth for low P supply and resulted on the row in 11.2, 9.8, 5.8, and
168 2.8; and at 30 cm distance from the row in 11.5, 11.3, 6.9 and 2.2. These values were lower
169 than those for moderate P supply treatment (25.7, 20.2, 7.7, 6.6 and 10.7, 10.9, 7.5, 3.4,
170 respectively).

171 Plant traits evaluated in this work are separated in four groups: nitrogen fixation, yield
172 components, phenological traits, and photosynthetic traits. Nitrogen fixation was studied by
173 isotope analysis. Dried grain samples were finely ground using a ball-mill, 2.5 mg of each
174 sample were weighed out using a microbalance and packed in tin capsules. These samples were
175 sent to UC Davis Stable Isotope Facility (Davis, USA) for ^{12}C, ^{13}C and ^{14}N, ^{15}N isotope

analyses. The percentage of N derived from the atmosphere (%Ndfa) was determined using the ^{15}N natural abundance method (Shearer and Kohl, 1986; Polania et al. 2016b). DOR 364 NN was used as a non-fixing reference plant.

$$\%\text{Ndfa} = \frac{\delta 15\text{N non fixing reference plant} - \delta 15\text{N of N2 fixing legume}}{\delta 15\text{N non fixing reference plant} - \beta} \text{x}100$$

Where β is the $\delta^{15}N$ value from the nitrogen fixing bean plant grown in N free medium. The β value used was –2.44 ‰ for grain at harvest (N. Barbosa, unpublished data). The β value was determined from a pot experiment in the greenhouse at CIAT following the procedure of Unkovich et al. (1994). Total seed N content per unit area (SDN_ha) was calculated using the values of N concentration in seed and dry weight of seed per area, seed C to N ration (SDCN) was calculated from the C and N concentrations. Total N derived from atmosphere or soil per unit area (Ndfa_ha and Ndfs_ha, respectively) were determined based on %Ndfa / %Ndfs and SDN_ha (Polania et al. 2016b). The following calculations were used, SDN_ha = (YDHA × SDN/100); Ndfa_ha= (SDN_ha × %Ndfa/100); Ndfs_ha= SDN_ha - Ndfa_ha.

Yield and yield components were measured as dry weight of grain yield (YDHA, humidity adjusted to 14 %) in kg ha^{-1}, pod number (PNA) and seed number per area (SDNA) as number per m^{-2}, 100 seed weight (100SDW) in g 100 $seeds^{-1}$; shoot biomass (SHBH) and stem biomass (SBH), and pod biomass at harvest (PBH) in kg ha^{-1}, seed carbon content (SDC) in % , and pod harvest index (PHI) was determined according to Beebe et al. (2013).

196 Phenological traits days to flowering (DF) and days to maturity (DPM) were evaluated.
197 Investigated photosynthetic traits were SPAD chlorophyll meter reading in younger fully
198 expanded leaf (SCMR) in SPAD units, stomatal conductance on younger fully expanded leaf
199 (SCOND) in mmol m^{-2} s^{-1}, photosynthetic efficiency on younger fully expanded leaf (FVFM)
200 as fv`/ fm`, and carbon isotope discrimination (CID) in ‰ (Beebe et al. 2013; Dhanapal et al.
201 2015; Polania et al. 2016a). Additional information about the traits can be found in the "Trait
202 Dictionaries for Fieldbook Development" at http://mbp.generationcp.org and
203 http://www.cropontology-curationtool.org/.

204

205 *Phenotypic data analysis*

206 Phenotypic data were analyzed using analysis of variance, Pearson's correlations between
207 traits, and principal component analysis (PCA), using the software SAS, v 9.3 (SAS-Institute
208 2011). Data shown in this work are adjusted means, correlation analyses were carried out using
209 all three replications.

210

211 *QTL detection*

212 The genetic Map of the D×B population used in this study was previously described, the
213 utilized genetic map of D×B has 291 markers (22 AFLP, 98 RAPD, 160 SSR and 11 gene-
214 based markers) (Galeano et al. 2011). For QTL analysis, two RILs were eliminated due to low
215 marker quality. Identification of significant QTLs was carried out using composite interval
216 mapping analysis of the program QTL Cartographer v. 1.21 (Wang et al. 2012) and thresholds
217 for the QTLs for each trait were determined by the generation of 1000 permutations.

Designated genomic regions that proved to be significant in the analysis were displayed using the program MapChart (Voorrips 2002).

Results

Phenotypic evaluation of the DxG RIL population in contrasting P conditions

SNF and yield traits were investigated under moderate and low P supply conditions in the DOR 364 × BAT 477 (D×B) population in a replicated field trial. Low (LP) and moderate soil P (MP) levels were generated by applying 10 and 30 kg P ha^{-1}, respectively, which reflects levels of P fertility found in farmer's fields, rather than extreme experimental conditions.

An overview of phenotypic data shows that yield component traits were strongly superior under MP conditions compared to LP environment (Table 2, Supplementary Fig. S1), all showing significant differences between P levels. An increase in P supply from LP to MP increased grain yield (from 599 to 1250 kg ha^{-1}), pod number per area (from 125 to 179 pods m^{-2}), seed number per area (from 587 to 949 seeds m^{-2}), and pod harvest index (from 72.6 to 74.3%). While yield was reduced by more than half in LP, seed carbon concentration (SDC) was the only trait slightly elevated in LP. BAT 477 was superior to DOR 364 under both conditions for nearly all traits.

Whereas seed N content per area was reduced from 44 to 21 kg ha^{-1} in LP, notably, there was no significant difference in %Ndfa between environments. Significant variation for %N derived from the atmosphere (%Ndfa) was observed, ranging from 8% to 42% and 11% to 43% in LP and MP conditions, respectively. In both environments parental line BAT 477 and DOR 364 maintained near mean values. Additionally, all photosynthetic traits measurements were

significantly different between two P environments, with the exception of CID. Intriguingly P stress delayed flowering time (DF) by about one day, but had no significant effect on days to physiological maturity (DPM).

In the majority of traits evaluated, performance in MP was correlated with performance in LP (Table 2). Strongest correlations were observed for DF, DPM, SCMR_F (SPAD chlorophyll meter reading at flowering) and 100SDW (100 seed weight). In some traits there was no correlation among RILs between MP and LP treatments even though several present significant variation among RILs in both treatments (e.g., %Ndfa, Ndfs_ha, SDN_ha) indicating environment-dependent traits with GxE interactions. Trait value distributions of the D×B RILs were continuous and normal for most traits in both P conditions (Supplementary Fig. S1), suggesting a quantitative inheritance. Transgressive segregation beyond the parental lines was observed for most traits, indicating promising combinations of parental alleles. Most pronounced positive transgressive segregation was observed for CID where most RILs displayed higher values of CID than the parental lines suggesting higher stomatal conductance and metabolic activity in these lines for effective use of water (Polania et al. 2016a).

Phenotypic correlations in contrasting P conditions

Under MP conditions, %Ndfa showed negative correlations with most shoot traits, pod number per area (PNA), seed number per area (SDNA), shoot biomass at harvest (SHBH) and pod biomass at harvest (PBH), and correlations with DF and DPM were also negative suggesting that large, high biomass, high yielding plants derived a smaller proportion of N from the

atmosphere (Table 3). N present in seeds of those plants is principally soil derived N (%Ndfs = 100 - %Ndfa), effective soil N uptake may have a larger variability dominating the correlation. Small, early maturing plants fixed proportionately more N from the atmosphere. In low P conditions the situation is different, %Ndfa is slightly but positively related to yield per hectare (YDHA), 100SDW and PHI, indicating that under LP stress atmospheric N fixation becomes a valuable asset. The values of Ndfa_ha and Ndfs_ha were calculated based on yield and seed N content, accordingly correlations observed were not independent. In LP the correlation between N concentration in seed SDN and %Ndfa was significant and negative. It is somewhat surprising to find that high N fixation is correlated with low N concentration suggesting that high N concentration may be more dependent on Ndfs. Correlations between seed N and seed C concentrations (SDN and SDC) were significant, and positive in both conditions, 0.37 and 0.34 for MP and LP, respectively

YDHA has high and significant correlations with yield component and biomass traits (PBH, SBH, SHBH, PNA and SDNA) in both P conditions. The pod and seed number per area and biomass traits, as expected, were highly correlated among each other, representing most of the highest observed trait correlations in Table 3. Under LP stress conditions YDHA correlated positively with PHI and 100SDW, supporting the importance of seed filling under stress, and negatively with DF and DPM.

Under LP treatment, CID was correlated positively with nearly all yield component traits, and negatively with %Ndfa, while under MP treatment CID was correlated negatively with 100SDW and SBH. Higher value of CID is an indicator of increased stomatal opening, gas

exchange and metabolic activity (reflected in a moderate correlation with stomatal conductance (SCOND) in both conditions). This is in line with the positive correlation on biomass and yield traits. CID data between MP and LP treatments were not significantly different (Table 2), however, significant differences between the RILs were observed in LP, indicating genetic variability among them.

Seed N derived from soil (Ndfs_ha) was more closely related to grain yield than the N derived from atmosphere (Ndfa_ha) under LP (r=0.82*** and 0.62***) and also at MP supply (r=0.91*** and 0.64***), respectively. Under LP conditions a group of ten RILs (RIE 89, 44, 87, 88, 65, 34, 37, 30, 82, 32) was identified with greater grain yield, and within them RIE 34, 30, 82 and 32 also combined higher values of %Ndfa in grain (Fig. 1). Among these ten RILs only RIE 32 had higher grain yield and also higher %Ndfa values under both low P and moderate P supply. A group of three RILs (RIE 32, 40, and 52) showed greater than mean values of grain yield and %Ndfa under both LP and MP conditions. Parental lines yielded generally poor with moderate values of %Ndfa under LP, with BAT 477 performing moderately better with MP conditions.

Principal component analysis

Principal component analysis was carried out to further investigate trait associations. With LP, component 1, which has mainly contributions of yield components, explained 25% of the total variability (Supplementary Table S1). A second component based mainly on N related traits (SDN, SDCN, %Ndfa and Ndfa_ha) explained 15% of the total variability. Although %Ndfa

306 correlated with yield in LP, this relationship did not emerge in the PCA. Under MP, component
307 1 was attributed mainly to yield components that explained 29% of total variance. A second
308 component, explaining 13% of total variation, was related with 100SDW, and photosynthetic
309 traits such as SCMR and CID. In MP the N fixation traits formed a third component with little
310 association with other traits. Principal component derived trait clustering is in line with
311 correlation results above (Supplementary Fig. S2). Looking at first two PCs, yield and biomass
312 traits appear as one cluster under both P treatments. This trait cluster also showed highest
313 correlations (Table 3). DF and DPM were closely linked in LP, but not in MP, whereas
314 correlation was high in both environments.

315

316 *Marker trait association analysis*

317

318 The linkage map of DOR 364 × BAT 477 used here contains 290 markers, mapped to 11
319 linkage groups covering a total distance of 1714 cM (Fig. 2, Galeano et al. 2011). Average
320 distance between markers was 6.3 cM, which is suitable for QTL identification. Composite
321 interval mapping analysis identified 55 QTL for 16 traits on nine linkage groups (Table 4).

322 QTL %Ndfa7.1^{DB} was identified on the lower arm of chromosome Pv07 explaining 21 % of
323 the phenotypic variance, DOR 364 contributing the positive allele. This QTL also explains 18
324 % of the Ndfa_ha variability, followed Ndfa_ha8.1^{DB} that explains 14 %. Both N concentration
325 and content have QTL on Pv02 and the upper arm of Pv07, in both cases BAT477 contributes
326 the positive allele. Taken together N related traits have QTL in 4 regions from both parental
327 genotypes, with those on Pv07 observed only in LP and Pv02 in MP, combinations of which
328 may explain the transgressive segregation.

For yield and yield component traits 26 QTLs were found; the majority in LP stress conditions (14 vs 12). YDHA QTL were discovered on Pv02 and Pv07 explaining 17 % and 19 % of the phenotypic variance with BAT 477 contributing the positive allele. Further yield component QTLs were found in six of the eleven chromosomes of the common bean genome. A QTL hotspot for yield components is observed on Pv02 composed of QTL SDNA2.1, PNA2.1 and 100SDW2.1, the latter has an opposing additive effect, hence the DOR 364 allele confers higher seed and pod number as well as lower seed weight. SBH2.1^{DB} and SCMR-m2.1^{DB} also localize in this QTL hotspot.

Eight QTL for 100SDW were found on three chromosomes, 100SDW6.1^{DB} and 100SDW6.2^{DB} appeared in both P conditions. In the same region SCMR-m6.2^{DB} is also observed in both environments, co-localizing with further QTL PNA6.1^{DB}, SDNA6.1^{DB}, SDNA6.2^{DB}, SDC6.2^{DB}, CID6.2^{DB} and SCMR-f6.3^{DB}. SDNA6.1 and PNA6.1, are powered by the opposing BAT 477 allele, accordingly SCMR and 100SDW are consistently positively correlated traits, whereas SDNA and PNA are negatively correlated with 100SDW in MP. CID is positively correlated with both PNA and SDNA in LP, where QTL CID6.1 was detected, and correspondingly also based on the BAT 477 allele. Taken together this constitutive QTL hotspot on Pv06 is likely caused by one gene that affects several traits. In addition to the Pv06 locus, which is the most stable QTL hotspot in this population, also100SDW9.2^{DB}, DF11.1 ^{DB}, SCMR-f4.1^{DB} and SCMR-m6.2^{DB} represent constitutive condition-independent QTL that are detected in both environments. Constitutive QTL found in LP and MP conditions are likely to be stable in other environments and can be exploited for marker assisted breeding.

Discussion

Symbiotic N fixation in low soil fertility

This study investigated the effect of low and moderate soil P stress on N fixation and yield traits in the population DOR 364 × BAT 477. Difference in soil P availability is not as drastic as in previous studies conducted under greenhouse (Miguel et al. 2013), hydroponics (Silva et al. 2014a), or growth media (Jiang et al. 2007) conditions, but rather reflects realistic levels found in farmer's fields, where P deficiency is commonly limiting yield (Lynch and Beebe 1995). Very low P levels would not represent bean production areas as bean crop would not be grown on such soils. Average yields in MP (1250 kg ha^{-1}) and LP (599 kg ha^{-1}) bracket average national yields of most developing countries (Beebe 2012) and can be considered representative of bean yields in the tropics. Hence data on well performing lines and QTL for yield and N related traits should be transferable to breeding programs.

%Ndfa values correlated positively with yield traits under LP stress indicating that this process aids plant performance under stress. Significant genetic variation in the D×B population for %Ndfa was observed, which has previously been reported specifically for roots and development of nodules (Bourion et al. 2007), furthermore Vadez et al. (1999) reported genotypic differences for SNF under LP due to differences in P use efficiency. Transgressive segregation was observed for most SNF and yield related traits. RIE 57 and 43 had excellent %Ndfa values in both trials, RIE 32, 40 and 52 combined good %Ndfa values with above average yield in both conditions (Fig. 1) and may be used in breeding for this trait.

374 An intriguing observation is that, while the MP treatment resulted in greater values of Ndfa_ha,
375 P levels did not have a significant effect on %Ndfa. However, seed N concentration was higher
376 in MP, indicating that N uptake from either source works more efficiently with sufficient P. Under
377 LP treatment plants were smaller, likely having a smaller root system to take up N from soil or
378 atmosphere. Previous studies showed that abiotic stress conditions such as high temperatures,
379 water stress and low soil fertility reduce nodulation and SNF (Hungria and Vargas, 2000; Polania
380 et al. 2016b). There is a substantial need for P in the N_2 fixation process (Tsvetkova and Georgiev
381 2007), because P is used during nodule formation and N fixation (Olivera et al. 2004; Singh 2015).
382 P levels in nodules were reported to be directly correlated to nodule activity and N fixation levels
383 (Rotaru and Sinclair 2009) and increase in P supply promoted N fixation (Leidi and Rodriguez-
384 Navarro 2000). The lack of significant difference in %Ndfa between MP and LP environments
385 may mean that the reported effect is too small to be observed in the low P conditions used here
386 or that in this experiment N was not limiting.

387 %Ndfa and SDN showed a surprising negative correlation of -0.44*** in LP. Hence, the converse
388 soil derived N (Ndfs) is positively correlated with seed N content, and has more variability and a
389 larger effect than Ndfa. Alternatively SNF may only be activated in severe N shortage, an N
390 uptake problem which is insufficiently alleviated by atmospheric N fixation. A positive
391 correlation of %Ndfa with yield suggests no problems in plants with high %Ndfa, favoring the
392 first option. SDN is negatively correlated with yield component traits in both conditions which
393 is likely a dilution effect from carbohydrate production, whereby seed N concentration would
394 be reduced by greater accumulation of carbohydrates in seed.

395 SDN and SDC are significantly positively correlated, which may seem counterintuitive given that
396 protein and carbohydrates constitute the seed's major components. However, protein actually has

a higher C content (>50 %) than carbohydrates (~44 %). Hence, RILs with superior N remobilization and increased protein content display higher SDC, whereas variation in carbohydrate levels hardly affects SDC, which averages ~43 %.

Indicators of N fixation

Several N fixation indicators have been reported in common bean and these include lateral root nodules, number of nodules, plant biomass, total plant N and grain yield (Bliss 1993). Evaluating plant biomass at harvest and yield in this experiment, these trends were confirmed for yield under LP stress. However, biomass traits are actually negatively correlated with %Ndfa in MP, hence SNF indicators based on per cent N fixed cannot be generally applied to predict SNF in all conditions. Isotope analysis to determine %Ndfa and Ndfa_ha may be necessary for meaningful information on SNF.

QTL evaluation for SNF related traits

In this work a genetic characterization of SNF and yield traits under P stress conditions was carried out, to add to several yield, phenological and root trait QTL that have been identified previously in the D×B population (Asfaw and Blair 2012; Blair et al. 2012a; Asfaw et al. 2012b). In this study a QTL for %Ndfa was found on chromosome 7, explaining 21% of the phenotypic variation under LP conditions. The QTL for seed N concentration SDN7.1 ^{DB} was

found on the opposing chromosome arm and two more at the end of Pv02 SDN2.1/2 ^{DB}. In these QTLs BAT 477 alleles support SDN and reduce %Ndfa. Kamfwa et al. (2015) evaluated a subset of the Andean Diversity Panel of 259 Andean bean genotypes in greenhouse and field experiments, reporting 26 significantly associated SNPs for SNF-related traits in 11 loci. The large number of associated loci, is largely due to a higher number of traits and higher variability in the studied panel of landraces, cultivars and breeding lines. QTLs for seed %Ndfa were reported on Pv02, 03 and 09. A marker associated with % N in seed on Pv02 is located ~3 MB from SDN2.1^{DB}, these may be the same locus. Ramaekers et al. (2013) performed evaluations in the RIL population G2333 × G19839 and identified QTL for %Ndfa in chromosomes Pv01, Pv04 and Pv10, indicating that other loci control phenotypic variation in that population. Consensus between genetic studies are few, suggesting that a larger number of loci is responsible for the observed genetic variability.

Analysis of yield components

Yield components of seed and pod number per area have high correlations among themselves and cluster closely in PCA analysis, showing that biomass at harvest and solid vegetative development is an important basis for yield. Only in MP conditions 100SDW and PHI do not form part of this correlated group. LP severely limits yield, reducing the means by more than half. Mourice and Tryphone (2012) showed that low P reduces biomass traits and that BAT 477 excels in pod and seed yield in seven tested genotypes. In the data presented here BAT 477 yields in LP are inferior to the mean of RILs but superior to DOR 364. Silva et al. (2014) evaluated 20 genotypes, finding that both DOR 364 and BAT 477 had poor leaf area under severely limited P conditions. Only

441 BAT 477 was above average in less severe stress, which is in agreement with current results.
442 Figure 1 indicates significant transgressive segregation for yield, hence improvement under these
443 conditions can be expected through breeding, using RIE87 which was among the best yielding
444 lines in both conditions, or RIE32 that combines good yields with above average value of %Ndfa.

445

446 *QTL analysis for yield component traits*

447

448 Three QTL were detected for grain yield, YDHA2.1^{DB} under LP, YDHA7.1^{DB} and nearby
449 YDHA7.2^{DB} in LP and MP conditions, respectively. The BAT 477 allele at these QTL led to
450 a yield advantage ranging from 37 to 97 kg/ha, representing ~6-7% of the RIL means in
451 respective environments. A QTLs hotspot around 100SDW6.1^{DB} and 100SDW6.2^{DB} was
452 detected in both P conditions, explaining the highest percentages of observed variation. QTLs
453 for seed weight associated to the same marker as 100SDW6.2$^{AG, DB}$ have been reported in
454 drought and under irrigation, in the DOR 477 × BAT 477 and A 55 × G122 populations
455 (Chavarro and Blair 2010; Blair et al. 2012a). In this study, two more environments (low and
456 moderate soil P conditions) demonstrate that 100SDW6.2$^{AG, DB}$ is a constitutive QTL whose
457 expression does not depend on the environment. Five traits showed two QTL each on
458 Chromosome 6, around 70 and 80 cM (100SDW, CID, SCMR-f, SCMR-m, SDNA6.1), we
459 hypothesize that this may be only one locus, split during the analysis by e.g. an imperfect genetic
460 map. SW2.1^{BR}, a QTL for 100SDW that was reported in the population of Buster/Roza (BR)
461 (Trapp et al. 2015), localized close to 100SDW2.1^{DB}. These may represent the same QTL
462 expressed in different environments and populations.

Yield markers detected in QTL studies have been notoriously inconsistent, rarely reproducible over years (Blair et al. 2012a) or hard to find at all (Ramaekers et al. 2013). Hence, the yield QTLs in this study may or may not prove to be useful for marker assisted selection (MAS). The two most promising loci for MAS appears to be at Pv07 since QTL for YDHA were found in both LP and MP environments, and the constitutive QTL cluster on Pv06 including yield components.

Nomenclature issues

In the course of the QTL analysis nomenclature conflicts arose. Blair et al. (2012) assigned 4 names to QTLs *Sw6.6*, *Sw6.10*, *Sw6.12* and *Sw6.15* in the same region in different conditions and years. We suggest this is the same QTL identified in this study as 100SDW6.6^{DB} in both P conditions, and we propose to assign only one name to a QTL if it is detected on the same locus in different conditions. We attempt to adhere to the trait definitions kept at cropontology.org, developed by Common Bean Community members in cooperation with IBP (Integrated Breeding Platform), in which the trait 100 seed weight is abbreviated as 100SDW. However, this trait was earlier published as Sw, hence we suggest to retain published QTL names, while using the ontology derived names for new QTL. Furthermore, SCMR-m6.2^{DB} is likely identical to Scr_PALDS, Scr_PALNS and Scr_KASNS (Asfaw et al. 2012b), representing a constitutive QTL for SPAD chlorophyll meter reading. As many ontology defined traits have longer abbreviations, which are in conflict with the BIC system that suggests 2-3 letter abbreviation (Miklas and Porch 2010), nomenclature rules need to be reviewed.

Low P stress shows similarities to drought stress

PHI was reported to be highly correlated with grain filling and yield, being of particular importance in stress conditions (Beebe et al. 2013; Rao 2014). A significant correlation of PHI with yield and 100SDW was found in LP stress, which may represent an effect on grain filling. This is in line with the importance of grain filling traits under stress reported in drought conditions (Assefa et al. 2013). In LP conditions yield, seed and pod number per area and biomass traits were significantly negatively correlated with DF and DPM, indicating that stress avoidance by early maturity combined with greater physiological efficiency (i.e., more yield per day; Polania et al. 2016a) is an important tolerance mechanism. This is again similar to observations in drought stress conditions underlining the similar mechanism for tolerance to these different stress conditions (Beebe et al. 2013). Fast maturing lines that dedicate resources to seed are most productive, however, drought stress alike, high biomass production at harvest is correlated with yield, which indicates that greater stem reserves may also be important to achieve higher seed yield under LP conditions. Successful genotypes combine good early biomass production with early maturity, while photoassimilates are directed efficiently to fill grain under stress.

A total of three QTL for PHI were identified, all in MP conditions. The supporting alleles originate from the higher yielding parental genotype BAT 477 in all cases. Asfaw et al. (2012) presented a QTL at same marker tagging QTL PHI5.2 ^{DB} for pod partitioning index and harvest index in drought in the same population, hence PHI5.2 ^{DB} appears to be another stable QTL.

Eight QTL for DF were identified, mostly under MP, the largest number of all traits. Likewise, Blair et al. (2012) reported almost the same number of QTL, nevertheless, only one QTL, $DF11.1^{DB}$, was consistent between both studies. Taken together, several QTL detected in this study were previously reported in other environments and populations, verifying the findings and indicating that these alleles will be useful for genetic improvement of yield traits in other backgrounds and environments.

Conclusions

Field experiments in moderate P and low P conditions demonstrated that low P severely limits yield. Transgressive segregation for yield was observed in the DOR 364 × BAT 477 population in low P stress and moderate P stress conditions. Investigation of symbiotic N fixation showed no significant difference in % N derived from atmosphere between environments, but D×B RILs revealed significant genetic variation in this trait. %Ndfa presented a modest positive correlation with productivity in LP conditions, but independent of general tendencies, some RILs that derived more N from fixation also yielded well in either LP or MP. Thus, good yield and superior N fixation can be combined. QTLs for %Ndfa and SDN were discovered on chromosomes 2 and 7. Low P stress resembles drought stress to some extent, as PHI, seed fill and early maturity are associated with tolerance to low P stress. QTL for yield and yield components were found that may be used in molecular breeding.

Acknowledgments

This project was supported by CGIAR Research Program on Grain Legumes. We would like to thank all donors who supported this work through their contributions to the CGIAR Fund. We

529 acknowledge the contributions made by E. Tovar, G. Borrero and M. Rivera and the Bean
530 Breeding team of the Bean Program of the International Center for Tropical Agriculture (CIAT).
531 We also thank M. Otero and J. Molina for technical assistance.

532

533 **Conflict of Interest:**

534 The authors declare that they have no conflict of interest.

535

References

Andrews M, Lea PJ (2013) Our nitrogen “footprint”: the need for increased crop nitrogen use efficiency. Annals of Applied Biology 163:165–169. doi: 10.1111/aab.12052

Araújo SS, Beebe S, Crespi M, et al (2015) Abiotic stress responses in legumes: Strategies used to cope with environmental challenges. Critical Reviews in Plant Sciences 34:237–280. doi: 10.1080/07352689.2014.898450

Asfaw A, Blair MW (2012) Quantitative trait loci for rooting pattern traits of common beans grown under drought stress versus non-stress conditions. Molecular Breeding 30:681–695. doi: 10.1007/s11032-011-9654-y

Asfaw A, Blair MW, Struik PC (2012) Multienvironment quantitative trait loci analysis for photosynthate acquisition, accumulation, and remobilization traits in common bean under drought stress. G3: Genes, Genomes, Genetics 2:579–595. doi: 10.1534/g3.112.002303

Assefa T, Beebe SE, Rao IM, et al (2013) Pod harvest index as a selection criterion to improve drought resistance in white pea bean. Field Crops Research 148:24–33. doi: 10.1016/j.fcr.2013.04.008

Batjes N (1997) A world data set of derived properties by FAO-UNESCO soil unit for global modelling

Batjes NH (2011) Global distribution of soil phosphorus retention potential. Wageningen, Plant Research International (PRI), Wageningen UR and ISRIC Worl Soil Information. Report 6, 42

Beebe S (2012) Common bean breeding in the tropics. In: Janick J (ed) Plant Breeding Reviews. pp 357–427

Beebe SE, Rao IM, Blair MW, Acosta-Gallegos JA (2013) Phenotyping common beans for adaptation to drought. Frontiers in Physiology 4:1–20. doi: 10.3389/fphys.2013.00035

Beebe SE, Rao IM, Cajiao C, Grajales M (2008) Selection for drought resistance in common bean also improves yield in phosphorus limited and favorable environments. Crop Science 48:582–592. doi: 10.2135/cropsci2007.07.0404

Beebe SE, Rojas-Pierce M, Yan X, et al (2006) Quantitative trait loci for root architecture traits correlated with phosphorus acquisition in common bean. Crop Science 46:413–423. doi: 10.2135/cropsci2005.0226

Blair MW, Galeano CH, Tovar E, et al (2012) Development of a Mesoamerican intra-genepool genetic map for quantitative trait loci detection in a drought tolerant × susceptible common bean (*Phaseolus vulgaris* L.) cross. Molecular Breeding 29:71–88. doi: DOI 10.1007/s11032-010-9527-9

Bliss FA (1993) Breeding common bean for improved biological nitrogen fixation. Plant and Soil 152:71–79

Bourion V, Laguerre G, Depret G, et al (2007) Genetic variability in nodulation and root growth affects nitrogen fixation and accumulation in pea. Annals of Botany 100:589–598. doi: 10.1093/aob/mcm147

Broughton WJ, Hern G, Blair M, et al (2003) Beans (*Phaseolus* spp.) – model food legumes. Plant and Soil 55:55–128

Chavarro MC, Blair MW (2010) QTL Analysis and effect of the fin locus on tropical adaptation in an inter-gene pool common bean population. Tropical Plant Biology 3:204–218. doi: 10.1007/s12042-010-9058-x

CIAT (1988) Simbiosis Leguminosa-Rizobio; manual de métodos de evaluacón, selección y manejo agronómico. Seccion Microbiologia de suelos, programa de pastos tropicales y programa de fríjol. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia.

Cichy KA, Blair MW, Galeano CH, et al (2009a) QTL analysis of root architecture traits and low phosphorus tolerance in an Andean bean population. Crop Science 49:59–68

Cichy KA, Snapp SS, Blair MW (2009b) Plant growth habit, root architecture traits and tolerance to low soil phosphorus in an Andean bean population. Euphytica 165:257–268. doi: 10.1007/s10681-008-9778-2

Dhanapal AP, Ray JD, Singh SK, et al (2015) Genome-wide association study (GWAS) of carbon isotope ratio ($\delta^{13}C$) in diverse soybean [*Glycine max* (L.) Merr.] genotypes. Theoretical and Applied Genetics 128:73–91. doi: DOI 10.1007/s00122-014-2413-9

Galeano CH, Fernandez AC, Franco-Herrera N, et al (2011) Saturation of an intra-gene pool linkage map: towards a unified consensus linkage map for fine mapping and synteny analysis in common bean. PloS one 6:e28135

Hall AE, Richard A, Condon AG, et al (1994) Carbon isotopo discriminations and plant breeding. In: Plant Breeding Reviews, Janick, Ju. John Wiley & Sons, Inc., pp 81–113

Hungria M, Vargas MAT (2000) Environmental factors affecting N2 fixation in grain legumes in the tropics, with an emphasis on Brazil. Field Crops Research 65:151–164. doi: 10.1016/S0378-4290(99)00084-2

Jiang C, Gao X, Liao L, et al (2007) Phosphate starvation root architecture and anthocyanin accumulation responses are modulated by the gibberellin-DELLA signaling pathway in Arabidopsis. Plant physiology 145:1460–70. doi: 10.1104/pp.107.103788

Kamfwa K, Cichy KA, Kelly JD (2015) Genome-wide association analysis of symbiotic nitrogen fixation in common bean. Theoretical and Applied Genetics. doi: 10.1007/s00122-015-2562-5

Leidi EO, Rodriguez-Navarro DN (2000) Nitrogen and phosphorus availability limit N fixation in bean. New Phytologist 147:337–346

Lynch JP, Beebe SE (1995) Adaptations of Beans (*Phaseolus vulgaris* L.) to low-phosphorus availability. HortScience 30:1165–1171

Marschner H (1995) Mineral Nutrition of Higher Plants, 2nd Ed. Academic Press Inc., London, UK

Miguel MA, Widrig A, Vieira RF, et al (2013) Basal root whorl number: A modulator of phosphorus acquisition in common bean (*Phaseolus vulgaris*). Annals of Botany 112:973–982. doi: 10.1093/aob/mct164

Miklas PN, Porch TG (2010) Guidelines for common bean QTL nomenclature. Annu Rep Bean Improv Coop 53:202–204

Mourice SK, Tryphone GM (2012) Evaluation of common bean (*Phaseolus vulgaris* L.) genotypes for adaptation to low phosphorus. International Scholarly Research Network Agronomy ID309614:1–9. doi: 10.5402/2012/309614

Olivera M, Tejera N, Iribarne C (2004) Growth, nitrogen fixation and ammonium assimilation in common bean (*Phaseolus vulgaris*): effect of phosphorus. Physiologia Plantarum 121:498–505. doi: 10.1111/j.1399-3054.2004.00355.x

Polania J, Poschenrieder C, Beebe S, Rao IM (2016a) Effective use of water and increased dry matter partitioned to grain contribute to yield of common bean improved for drought

resistance. Frontiers in Plant Science. doi: 10.3389/fmicb.2015.00927

Polania J, Poschenrieder C, Beebe S, Rao IM (2016b). Estimation of phenotypic variability in symbiotic nitrogen fixation ability of common bean under drought stress using ^{15}N natural abundance in grain. European Journal of Agronomy 79: 66–73. doi:10.1016/j.eja.2016.05.014

Polanía J, Rao M, Beebe S, Garcia R (2009) Root development and distribution under drought stress in common bean (*Phaseolus vulgaris* L.) in a soil tube system. Agronomia Colombiana 27:25–32

Ramaekers L, Galeano CH, Garzón N, et al (2013) Identifying quantitative trait loci for symbiotic nitrogen fixation capacity and related traits in common bean. Molecular Breeding 31:163–180. doi: 10.1007/s11032-012-9780-1

Ramaekers L, Remans R, Rao IM, et al (2010) Strategies for improving phosphorus acquisition efficiency of crop plants. Field Crops Research 117:169–176

Ramírez M, Flores-Pacheco G, Reyes JL, et al (2013) Two common bean genotypes with contrasting response to phosphorus deficiency show variations in the microRNA 399-mediated PvPHO2 regulation within the PvPHR1 signaling pathway. International Journal of Molecular Sciences 14:8328–8344. doi: 10.3390/ijms14048328

Rao IM (2014) Advances in improving adaptation of common bean and Brachiaria forage grasses to abiotic stresses in the tropics. In: Pessarakl M (ed) Handbook of Plant and Crop Physiology, Third Edit. CRC Press, Taylor and Francis Group, Boca Raton, FL., pp 847–889

Rao IM, Miles JW, Beebe SE, Horst WJ (2016) Root adaptations to soils with low fertility and aluminium toxicity. Annals of Botany. doi: 10.1093/aob/mcw073

Remans R, Beebe S, Blair M, et al (2007) Physiological and genetic analysis of root

responsiveness to auxin-producing plant growth-promoting bacteria in common bean (*Phaseolus vulgaris* L.). Plant and Soil 302:149–161

Rotaru V, Sinclair TR (2009) Interactive influence of phosphorus and iron on nitrogen fixation by soybean. Environmental and Experimental Botany 66:94–99

SAS-Institute (2011) SAS Institute Inc. 2011. SAS® 9.3 System Options: Reference, Second Edition

Shearer G, Kohl DH (1986) N2-Fixation in Field Settings: Estimations Based on Natural 15N Abundance. Australian Journal of Plant Physiology 13:699–756. doi: 10.1071/PP9860699c

Silva DA da, Fátima Esteves AJ, Messias U, et al (2014) Efficiency in the use of phosphorus by common bean genotypes. Scientia Agricola 71:232–239

Singh A (2015) Nitrogen and phosphorus resorption efficiency in some leguminous and non-leguminous tropical tree species planted on coal mine spoil in a tropical dry environment. Ambit Journal of Educational Research and Reviews 1:1–7

Sponchiado BN, White JW, Castillo JA, Jones PG (1989) Root growth of four common bean cultivars in relation to drought tolerance in environments with contrasting soil types. Exp Agric 25:249–257

Trapp JJ, Urrea C A., Cregan PB, Miklas PN (2015) Quantitative trait loci for yield under multiple stress and drought conditions in a dry bean population. Crop Science 55:1596. doi: 10.2135/cropsci2014.11.0792

Tsvetkova GE, Georgiev GI (2007) Changes in phosphate fractions extracted from different organs of phosphorus starved nitrogen fixing pea plants. Journal of Plant Nutrition 30:2129–2140. doi: 10.1080/01904160701700616

668 Unkovich MJ, Pate JS, Sanford P, Armstrong EL (1994) Potential precision of the delta-N-^{15}
669 natural-abundance method in-field estimates of nitrogen-fixation by crop and pasture legumes
670 in south-west Australia. Australian Journal of Agricultural Research 45:119–132

671 Vadez V, Lasso JH, Beck DP, Drevon JJ (1999) Variability of N2-fixation in common bean
672 (*Phaseolus vulgaris* L .) under P deficiency is related to P use efficiency N2-fixation tolerance
673 to P deficiency. Euphytica 199079:231–242. doi: 10.1016/j.jplph.2012.03.013

674 Voorrips R. (2002) MapChart: Software for the graphical presentation of linkage maps and
675 QTLs. The Journal of heredity 93:77–78

676 Wang S, Basten CJ, Zeng Z-B (2012) Windows QTL Cartographer 2.5. Department of
677 Statistics, North Carolina State University, Raleigh, NC.(
678 http://statgen.ncsu.edu/qtlcart/WQTLCart.htm)

679

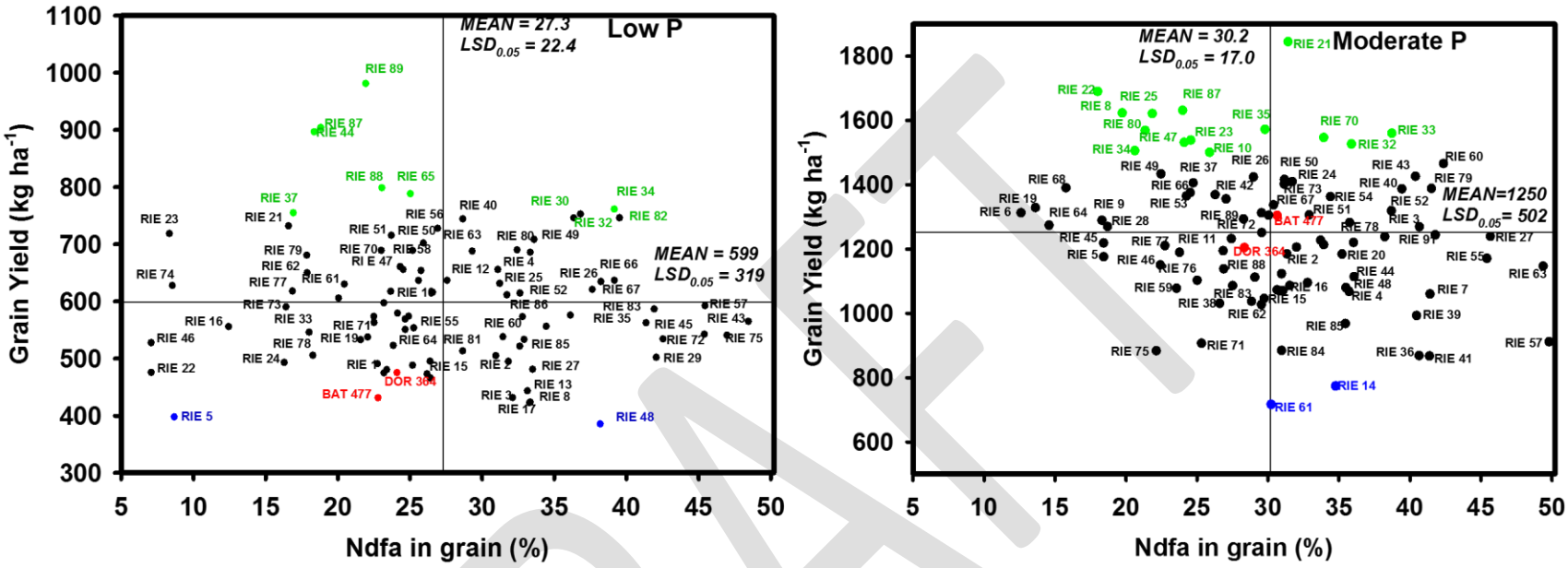
Figures

Fig. 1 Relationship between grain yield and N derived from atmosphere (%) in the DOR 364 × BAT 477 population evaluated under low and moderate P supply. Genotypes with best grain yield in green color; worst in dark blue and parental lines in red

Fig. 2 QTLs identified in the DOR 364 × BAT 477 population associated with phenotypic traits in moderate P and low P stress conditions. Bars represent QTL and color corresponds to the parent contributing the positive allele; DOR 364 blue and BAT 477 red

Figure

Click here to download Figure Fig. 1 Relationship between grain yield and N.docx



Figure

Click here to download Figure Fig. 2 QTLs identified map.docx

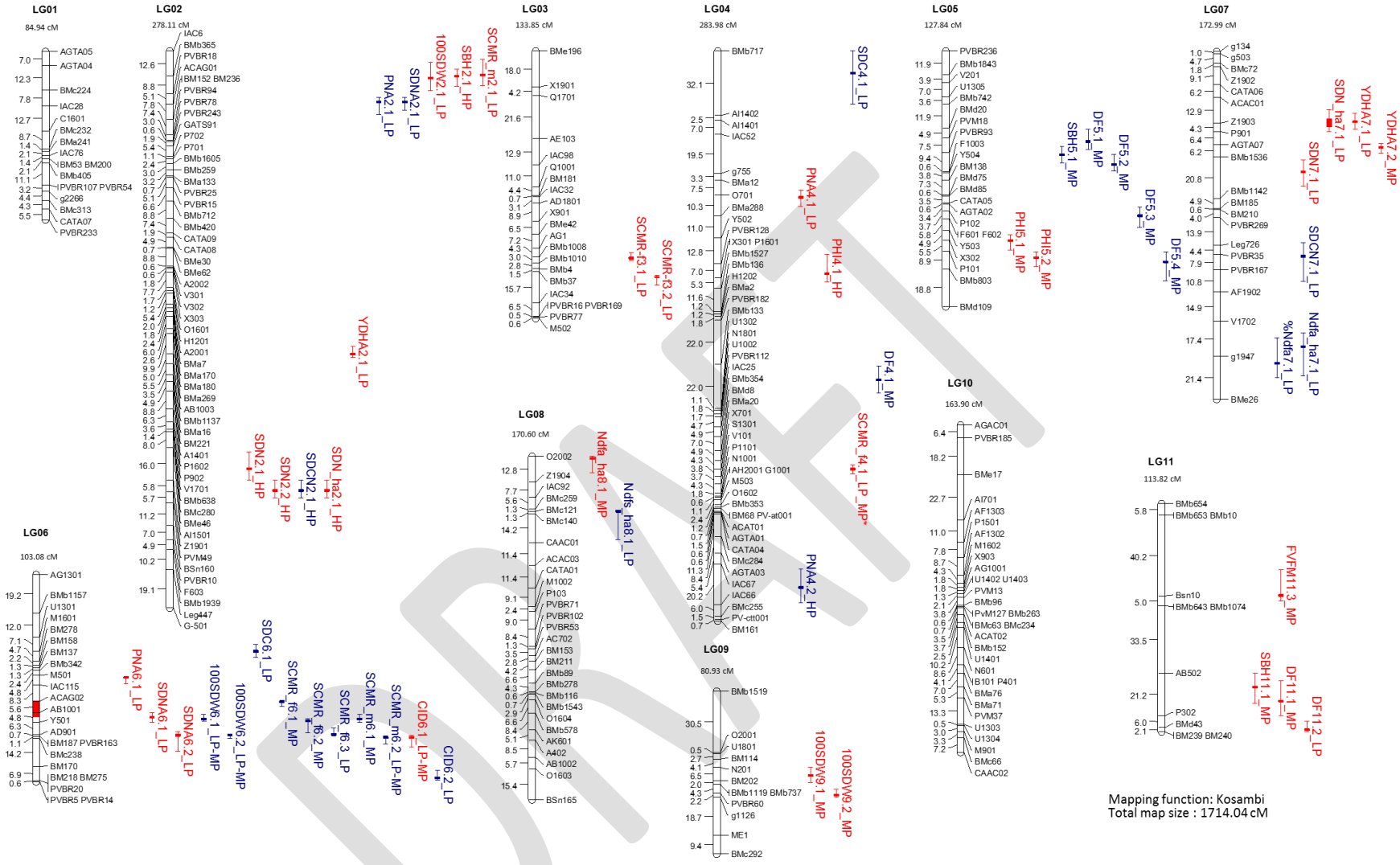


Table 1 Statistics of the DOR 364 × BAT 477 population and parental data under low and moderate soil phosphorus environments (LP and MP)

Type	Trait	Env.	DOR364	BAT477	Min.	Max.	Mean	Significance LP vs MP	Correlation LP vs MP	Between RILs
Nitrogen fixation	%Ndfa	LP	21.31	22.5	8.44	41.57	23.92	ns	ns	**
		MP	27.92	28.42	10.51	42.75	26.53			**
	Ndfa_ha	LP	3.63	3.79	1.93	10.14	5.32	***	ns	*
		MP	11.02	12.19	5.15	20.84	11.81			*
	Ndfs	LP	12.46	10.58	7.16	26.6	15.65	***	ns	**
		MP	28.23	31.41	14.85	50.74	32.94			***
	SDN	LP	3.45	3.28	3.06	3.85	3.48	***	0.28***	***
		MP	3.31	3.35	3.26	3.93	3.6			***
	SDN_ha	LP	16.51	14.17	12.62	40.34	21	***	ns	***
		MP	39.71	43.55	19.01	66.34	44.34			***
	SDCN	LP	12.29	13.03	11.11	14.11	12.35	***	0.29***	***
		MP	12.86	12.72	10.92	13.1	11.88			***
Yield components	YDHA	LP	475.45	431.69	385.68	981.35	599	***	0.14**	***
		MP	1204.81	1304.83	716.49	1845.01	1250			***
	PNA	LP	97.31	104.68	75.01	187.62	124.98	***	ns	***
		MP	182.02	171.58	124.74	260.13	179.02			ns
	SDNA	LP	454.4	544.33	326.23	914.68	586.61	***	0.12**	**
		MP	990.84	915.02	592.6	1494.22	949.4			ns
	100SDW	LP	18.4	21.92	15.2	23.07	18.74	***	0.60***	***
		MP	20.27	23.74	17.39	25.54	21.22			***
	PHI	LP	73.08	73.44	64.75	76.81	72.62	***	0.20***	***
		MP	73.54	76.69	70.42	79.44	74.28			***
	SHBH	LP	1146.52	1395.44	872.13	2351.79	1544.55	***	ns	*
		MP	2762.15	3130.55	1602.05	3858.91	2733.36			ns

	SBH	LP	200.08	296.61	121.72	452.43	250.77	***	0.20***	***
		MP	401.49	551.39	201.47	666.79	398.97			***
	PBH	LP	944.92	1099.85	751.97	1999.1	1293.84	***	ns	***
		MP	2359	2579.04	1379.91	3203.63	2334.31			Ns
	SDC	LP	42.31	42.46	41.94	43.41	42.81	***	ns	Ns
		MP	42.59	42.58	40.94	43.15	42.59			Ns
Phenological traits	DF	LP	39.52	41.72	35.15	41.64	39.39	***	0.46***	***
		MP	39.07	40.11	36.17	40.59	38.47			***
	DPM	LP	63.79	68.03	58.78	70.79	65.2	ns	0.58***	***
		MP	64.81	68.62	60.17	71.01	65			***
Photosynthetic traits	SCMR-f	LP	34.38	37.36	29.86	42.28	35.29	***	0.60***	***
		MP	36.79	37.2	31	41.3	36.65			***
	SCMR-m	LP	39.16	44.04	27.22	44.49	37.11	***	0.34***	***
		MP	39.7	47.32	26.24	47	40.08			***
	SCOND	LP	143.62	187.8	87.66	299.36	184.25	***	0.16**	***
		MP	286.06	219.13	150.01	401.84	245.49			Ns
	FVFM	LP	0.59	0.6	0.48	0.64	0.57	***	ns	Ns
		MP	0.66	0.61	0.53	0.68	0.6			ns
	CID	LP	19.63	19.3	18.76	21.11	19.95	ns	ns	***
		MP	19.73	19.35	9.43	20.86	19.86			ns

Min, Minimum value; Max, Maximum value; Significance between LP and MP evaluated by t-test for all traits, Pearson correlation of genotypes in MP and LP were calculated and significance of differences between RILs. ns, *, **, *** indicates no significance and significance at the 0.05, 0.01 and 0.001 probability levels. For full trait names see list of abbreviations

Table 2 Phenotypic correlations of RILs of the DOR 364 × BAT 477 population under low and moderate P environments. Correlations in moderate P are displayed in the upper right corner above diagonal and low P in lower left part

Variables	%Ndfa	Ndfa_ha	Ndfs_ha	SDN	SDN_ha	SDCN	YDHA	PNA	SDNA	100SDW	PHI	SHBH	SBH	PBH	CSD	DF	DPM	SCMR-f	SCMR-m	SCOND	FVFM	CID
%Ndfa		0.70***	-0.41***	ns	ns	ns	ns	-0.24***	-0.23***	ns	ns	-0.24***	-0.28***	-0.23***	-0.18**	-0.14*	-0.13*	0.26***	ns	0.20***	ns	ns
Ndfa_ha	0.85***		0.29***	-0.26***	0.55***	0.25***	0.64***	0.22***	0.25***	ns	ns	0.28***	0.22***	0.28***	ns	-0.24***	ns	0.25***	0.14*	ns	ns	ns
Ndfs_ha	-0.34***	ns		0.29***	0.88***	-0.28***	0.91***	0.63***	0.67***	ns	ns	0.71***	0.68***	0.70***	ns	ns	ns	ns	0.30***	-0.16**	0.13*	ns
SDN	-0.44***	ns	ns		ns	-0.96***	ns	-0.16**	-0.14*	ns	-0.19**	-0.15*	ns	-0.16**	0.34***	0.15*	ns	ns	ns	ns	ns	-0.20***
SDN_ha	0.12*	0.64***	0.92***	ns		ns	0.99***	0.59***	0.64***	ns	ns	0.68***	0.63***	0.68***	ns	-0.18**	ns	ns	0.30***	ns	ns	-0.13*
SDCN	0.42***	ns	ns	-0.98***	ns		ns	0.18**	0.15*	ns	0.16**	0.16**	ns	0.18**	ns	-0.14*	ns	ns	ns	ns	0.17**	0.19**
YDHA	0.20***	0.62***	0.82***	ns	0.98***	ns		0.62***	0.67***	ns	ns	0.7***	0.64***	0.70***	ns	-0.20***	ns	ns	0.31***	ns	ns	ns
PNA	ns	0.23***	0.52***	ns	0.54***	ns	0.54***		0.93***	-0.22***	ns	0.88***	0.79***	0.88***	ns	ns	ns	ns	0.16**	ns	0.12*	ns
SDNA	ns	0.25***	0.53***	-0.16**	0.55***	0.14*	0.57***	0.90***		-0.22***	ns	0.91***	0.80***	0.92***	ns	-0.14*	ns	ns	0.17**	-0.13*	ns	ns
100SDW	0.17**	0.24***	ns	-0.18**	0.21***	0.18**	0.23***	ns	ns		ns	ns	ns	ns	ns	-0.12*	0.25***	0.28***	0.30***	ns	ns	-0.21***
PHI	0.21***	0.22***	ns	-0.18**	0.13*	0.15*	0.16**	ns	ns	0.15*		ns	ns	ns	-0.13*	-0.20***	ns	ns	ns	ns	ns	ns
SHBH	ns	0.34***	0.57***	-0.18**	0.63***	0.17**	0.65***	0.87***	0.90***	0.19**	ns		0.93***	1***	ns	-0.17**	ns	0.12*	0.31***	-0.12*	0.12*	ns
SBH	ns	0.18**	0.57***	ns	0.56***	ns	0.55***	0.77***	0.79***	0.21***	-0.17**	0.91***		0.89***	ns	ns	0.19**	ns	0.32***	ns	0.14*	-0.16**
PBH	ns	0.36***	0.56***	-0.20***	0.63***	0.19**	0.65***	0.87***	0.91***	0.18**	ns	1***	0.87***		ns	-0.19**	ns	0.14*	0.30***	ns	0.12*	ns
CSD	-0.25***	-0.12*	0.20***	0.37***	ns	-0.23***	ns	ns	ns	ns	-0.13*	ns	ns	ns		ns	ns	ns	ns	ns	0.24***	ns
DF	-0.25***	-0.35***	-0.27***	0.25***	-0.40***	-0.25***	-0.44***	-0.31***	-0.33***	ns	ns	-0.33***	-0.20***	-0.36***	ns		0.51***	-0.19**	ns	ns	ns	ns
DPM	-0.23***	-0.23***	ns	0.19**	-0.20***	-0.18**	-0.25***	-0.2***	-0.22***	0.31***	ns	-0.13*	ns	-0.15*	ns	0.63***		ns	0.42***	ns	ns	ns
SCMR-f	-0.15*	ns	0.13*	ns	ns	ns	ns	ns	ns	0.13*	ns	ns	ns	ns	0.17**	-0.39***	ns		0.29***	ns	ns	ns
SCMR-m	ns	ns	ns	ns	0.14*	ns	ns	ns	ns	0.40***	ns	ns	ns	ns	ns	ns	0.36***	0.34***		-0.12*	ns	ns
SCOND	ns	ns	0.20***	ns	0.17**	ns	0.17**	0.12*	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns		-0.20**	0.14*
FVFM	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	-0.16**	ns	0.21***	ns		ns
CID	-0.22***	ns	0.20**	ns	ns	ns	0.12*	0.20***	0.26***	ns	ns	0.20***	0.17**	0.20***	ns	ns	ns	ns	ns	0.17**	ns	

For full trait names see list of abbreviations

Table 3 Significant QTL for Nitrogen related traits, yield components, phenological and photosynthetic traits under low P (LP) and moderate P (MP) environments in the DOR 364 × BAT 477 population

Trait	QTL name	Prev. name	Env.	Chr.	Marker	Position	LOD	R2	Additive	Source
Nitrogen fixation										
%Ndfa	%Ndfa7.1[DB]		LP	7	g1947	154.61	3.8	0.21	3.86	D
Ndfa_ha	%Ndfa_ha7.1[DB]		LP	7	V1702	147.3	3.34	0.18	0.78	D
	%Ndfa_ha8.1[DB]		MP	8	O2002	1	3.48	0.14	-1.34	B
Ndfs	Ndfs8.1[DB]		LP	8	BMc121	27.4	3.13	0.11	1.3	D
SDN	SDN2.1[DB]		MP	2	AI1501	208.3	4.38	0.2	-0.06	B
	SDN2.2[DB]		MP	2	Z1901	219.2	4.45	0.16	-0.06	B
	SDN7.1[DB]		LP	7	BMb1536	60	4.17	0.28	-0.09	B
SDN_ha	SDN_ha 2.1[DB]		MP	2	Z1901	219.2	4	0.16	-3.32	B
	SDN_ha 7.1[DB]		LP	7	Z1903	35.1	3.43	0.11	-1.39	B
SDCN	SDCN2.1[DB]		MP	2	Z1901	219.2	4.57	0.17	0.18	D
	SDCN7.1[DB]		LP	7	BMb1536	59	4.56	0.26	0.31	D
Yield components										
YDHA	YDHA2.1[DB]		LP	2	BMa16	151.2	4.88	0.17	-46.88	B
	YDHA7.1[DB]		LP	7	Z1903	35.1	3.17	0.1	-36.91	B
	YDHA7.2[DB]		MP	7	AGTA07	47.8	4.21	0.19	-97.3	B
PNA	PNA2.1[DB]		LP	2	PVBR18	25.4	5.09	0.17	10.27	D
	PNA4.1[DB]		LP	4	O701	73	5.62	0.19	-12.98	B
	PNA4.2[DB]		MP	4	IAC67	267.6	3.85	0.24	14.23	D
	PNA6.1[DB]		LP	6	M501	51.4	3.73	0.12	-8.56	B
SDNA	SDNA2.1[DB]		LP	2	PVBR18	25.41	3.22	0.14	55.52	D
	SDNA6.1[DB]		LP	6	AB1001	71.11	3.54	0.15	-53.37	B
	SDNA6.2[DB]		LP	6	PVBR163	80.21	3.99	0.15	-52.66	B
100SDW	100SDW2.1[DB]	SW2.1[BR(1)]	LP	2	BMb365	12.61	3.43	0.08	-0.52	B
	100SDW6.1[DB]	Sw6.6[DB (2)], Sw6.10[DB (2)], Sw6.12[DB (2)], Sw6.15[DB (2)]	MP	6	AB1001	72.11	4.86	0.24	1.06	D
			LP	6	AB1001	72.11	3.79	0.15	0.69	D
	100SDW6.2[DB]	*Sw6.1*[AG (3)], *Sw6.2*[AG (3)], *Sw6.3*[AG (3)]	MP	6	PVBR163	80.21	12.87	0.42	1.39	D
			LP	6	PVBR163	80.21	6.71	0.2	0.78	D
	100SDW9.1[DB]		MP	9	N201	41.8	4.01	0.1	-0.61	B
	100SDW9.2[DB]		LP	9	PVBR60	51.6	4.99	0.15	-0.61	B
			MP	9	PVBR60	51.6	4.12	0.11	-0.62	B
PHI	PHI4.1[DB]		MP	4	PVBR128	111	3.8	0.13	-0.52	B
	PHI5.1[DB]		MP	5	X302	94.6	4.26	0.13	-0.55	B
	PHI5.2[DB]		MP	5	P101	103.2	3.42	0.14	-0.57	B

SBH	SBH2.1[DB]		MP	2	BMb365	12.6	4.3	0.15	-40.71	B
	SBH5.1[DB]		MP	5	PVBR93	51.7	4.83	0.19	48.18	D
	SBH11.2[DB]		MP	11	AB502	91.5	4.48	0.19	-46.03	B
SDC	SDC4.1[DB]		LP	4	BMb717	11	3.39	0.2	0.11	D
	SDC6.1[DB]		LP	6	M1601	38.3	4.47	0.16	0.1	D
Phenological										
DF	DF4.1[DB]		MP	4	BMb133	167.11	4.21	0.35	0.68	D
	DF5.1[DB]		MP	5	PVM18	45.21	4.07	0.16	0.45	D
	DF5.2[DB]		MP	5	PVBR93	56.71	4.4	0.18	0.49	D
	DF5.3[DB]		MP	5	P102	82.21	4.41	0.16	0.46	D
	DF5.4[DB]		MP	5	P101	105.21	3.62	0.14	0.43	D
	DF11.1[DB]		MP	11	AB502	96.51	4.38	0.28	-0.6	B
	DF11.2[DB]	*Df11.1* [DB (2)]	MP	11	P302	108.71	3.6	0.18	-0.49	B
			LP	11	BMd43	111.71	4.64	0.14	-0.51	B
Photosynthetic										
SCMR-f	SCMR-f3.1[DB]		LP	3	BMb1008	103.31	6.27	0.16	-1.42	B
	SCMR-f3.2[DB]		LP	3	BMb37	112.51	5.39	0.19	-1.34	B
	SCMR-f4.1[DB]		MP	4	X701	208.51	3.92	0.12	-0.78	B
			LP	4	X701	208.51	3.31	0.08	-0.64	B
	SCMR-f6.1[DB]		MP	6	AB1001	73.11	7.4	0.28	1.2	D
	SCMR-f6.2[DB]		MP	6	ACAG02	63.41	5.39	0.2	1.02	D
	SCMR-f6.3[DB]		LP	6	Y501	79.91	5.63	0.15	0.9	D
SCMR-m	SCMR-m2.1[DB]		LP	2	IAC6	12.01	3.17	0.09	-1.37	B
	SCMR-m6.1[DB]		MP	6	AB1001	72.11	2.99	0.14	1.42	D
	SCMR-m6.2[DB]	Scr-PALDS [DB (4)], Scr-PALNS [DB (4)], Scr-KASNS [DB (4)]	MP	6	BMc238	81.41	7.85	0.26	1.92	D
			LP	6	BMc238	81.41	7.85	0.25	2.22	D
FVFM	FVFM11.3[DB]		MP	11	BMb10	45.8	3.8	0.14	-0.01	B
CID	CID6.1[DB]		LP	6	PVBR163	81.2	5.45	0.22	-0.24	B
	CID6.2[DB]		LP	6	BM170	101.5	3.22	0.14	0.22	D

Previous name is stated if a QTL in a similar location was published in (1) Trapp et al. 2015, (2) Blair et al. (2012), (3) Chavarro and Blair (2010), (4) Asfaw et al. (2012). Env: Environment, Chr: Chromosome, source states the origin of the positive allele from D DOR 364 parent or B BAT 477 parent. For full trait names see list of abbreviations